DESCRIPTION

PREVENTIVE/REMEDY FOR RESPIRATORY DISEASES

TECHNICAL FIELD

The present invention relates to prophylactic/therapeutic agents and diagnostic agents for respiratory diseases, screening of the prophylactic/therapeutic agents for respiratory diseases, etc.

BACKGROUND ART

As the smoking generation is aged and their average life expectancy is prolonged, chronic obstructive pulmonary disease, chronic bronchitis, pulmonary emphysema, diffuse panbronchiolitis, intrinsic asthma, etc. are considered to be major diseases in respiratory diseases in the future.

It is revealed that smoking can be an evident causative factor of chronic obstructive pulmonary disease. Obstructive disorders proceed by smoking, and the degree of the disorders depends on the number of cigarettes smoked. Specifically; the disorders proceed more easily as smoking is initiated at an early age. In addition, a dose correlation between smoking and bronchial gland hyperplasia has been confirmed.

In animal experiments, there are many reports that emphysematous change can be caused by smoking.

Physiological changes in chronic obstructive pulmonary disease (hereinafter abbreviated sometimes as COPD) are charaterized with unique abnormal findings observed in 3 regions, that is, central airway, peripheral airway, and lung parenchyma. In lesions in the central airway, hyperplasia of goblet cells and morphological change in secretary tissues such as growth and hypertrophy of cells in submucosal glands are observed. With respect to inflammatory cells, an increase in macrophages and activated T lymphocytes is indicated in the airway mucus. As lesions in the region of bronchiole, mucus plugging in the airway lumen, abnormal formation of goblet cells in the airway epithelium, infiltration of inflammatory cells in the airway wall, and thickening and fibrosis of smooth muscles are observed. In alveolar parenchyma, pulmonary emphysema lesions defined by destruction and disappearance of alveolus and expansion of the air space are observed. Imbalance between protease and antiprotease is considered involved in these lesions. Any of these physiological changes cause airway obstruction.

Cholesterol 25-hydroxylase (CH25H) (GenBank Accession NO. NM_003956) is one kind of cholesterol hydroxylase and has an activity of converting cholesterol into 25-hydroxycholesterol (25-HC) (J. Biol. Chem. 273:34316-34327 (1998)). The product 25-hydroxycholesterol is known to induce interleukin-β in macrophage (Eur. J. Clin. Invest. 32:35-42 (2002)).

Prostate differentiation factor (GDF15, PLAB) (GenBank Accession NO. AF003934) has an effect of promoting a neutrophil infiltration action via interleukin 8 (J. Immunol. 171:2057-2065 (2003)).

Matrix metalloproteinase 19 (MMP19) (GenBank Accession NO. U38321) is an enzyme having a proteolysis enzyme activity (J. Biol. Chem. 272:4281-4286 (1997)).

Under these circumstances, there is desire for the development of prophylactic/therapeutic agents for respiratory diseases (for example, chronic obstructive pulmonary disease etc.), which are excellent with less side effects, as well as diagnostic agents for respiratory diseases.

DISCLOSRUE OF THE INVENTION

In order to solve the problems described above, the present inventors made extensive studies and have found a gene whose expression is significantly increased or decreased in lung tissue in a patient with lung cancer with a complication of chronic obstructive pulmonary disease (COPD), and as a result of further examination on the basis of this finding, the present invention has been accomplished.

That is, the present invention relates to:

(1) A prophylactic/therapeutic agent for respiratory diseases, comprising a compound or its salt that inhibits the activity of a protein comprising the same or substantially the same amino acid sequence as the amino acid sequence represented by SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 30, SEQ ID NO: 32, SEQ ID NO: 34, SEQ ID NO: 36, SEQ ID NO: 38, SEQ ID NO: 40, SEQ ID NO: 42, SEQ ID NO: 44, SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 58, SEQ ID NO: 60, or SEQ ID NO: 62, its partial peptide, or a salt thereof.

(1a) A prophylactic/therapeutic agent for respiratory diseases, comprising a compound or its salt that inhibits the activity of a protein comprising the same or substantially the same amino acid sequence as the amino acid sequence represented by

SEQ ID NO: 2, its partial peptide, or a salt thereof.

(1b) A prophylactic/therapeutic agent for respiratory diseases, comprising a compound or its salt that inhibits the activity of a protein comprising the same or substantially the same amino acid sequence as the amino acid sequence represented by SEQ ID NO: 4, its partial peptide, or a salt thereof.

(1c) A prophylactic/therapeutic agent for respiratory diseases, comprising a compound or its salt that inhibits the activity of a protein comprising the same or substantially the same amino acid sequence as the amino acid sequence represented by SEQ ID NO: 30, its partial peptide, or a salt thereof.

(2) The agent according to (1), wherein the compound is a compound that inhibits the activity of a protein comprising the amino acid sequence represented by SEQ ID NO: 2, its partial peptide, or a salt thereof.

(3) A prophylactic/therapeutic agent for respiratory diseases, comprising a compound or its salt that inhibits the expression of a gene for a protein comprising the same or substantially the same amino acid sequence as the amino acid sequence represented by SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 30, SEQ ID NO: 32, SEQ ID NO: 34, SEQ ID NO: 36, SEQ ID NO: 38, SEQ ID NO: 40, SEQ ID NO: 42, SEQ ID NO: 44, SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 58, SEQ ID NO: 60, or SEQ ID NO: 62, its partial peptide, or a salt thereof.

(3a) A prophylactic/therapeutic agent for respiratory diseases, comprising a compound or its salt that inhibits the expression of a gene for a protein comprising the same or substantially the same amino acid sequence as the amino acid sequence represented by SEQ ID NO: 2, its partial peptide, or a salt thereof.

(3b) A prophylactic/therapeutic agent for respiratory diseases, comprising a compound or its salt that inhibits the expression of a gene for a protein comprising the same or substantially the same amino acid sequence as the amino acid sequence represented by SEQ ID NO: 4, its partial peptide, or a salt thereof.

(3c) A prophylactic/therapeutic agent for respiratory diseases, comprising a compound or its salt that inhibits the expression of a gene for a protein comprising the same or substantially the same amino acid sequence as the amino acid sequence represented by SEQ ID NO: 30, its partial peptide, or a salt thereof.

(4) The agent according to (3), wherein the compound is a compound that

inhibits the expression of a gene for a protein comprising the amino acid sequence represented by SEQ ID NO: 2, its partial peptide, or a salt thereof.

(5) An antisense polynucleotide comprising the entire or part of a base sequence complementary or substantially complementary to a base sequence of a polynucleotide encoding a protein comprising the same or substantially the same amino acid sequence as the amino acid sequence represented by SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 30, SEQ ID NO: 32, SEQ ID NO: 34, SEQ ID NO: 36, SEQ ID NO: 38, SEQ ID NO: 40, SEQ ID NO: 42, SEQ ID NO: 44, SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 58, SEQ ID NO: 60, or SEQ ID NO: 62, or its partial peptide.

(5a) An antisense polynucleotide comprising the entire or part of a base sequence complementary or substantially complementary to a base sequence of a polynucleotide encoding a protein comprising the same or substantially the same amino acid sequence as the amino acid sequence represented by SEQ ID NO: 2, SEQ ID NO: 4 or SEQ ID NO: 30, or its partial peptide.

(6) A pharmaceutical preparation comprising the antisense polynucleotide according to (5).

(6a) A pharmaceutical preparation comprising the antisense polynucleotide according to (5a).

(7) The pharmaceutical preparation according to (6), which is a prophylactic/therapeutic agent for respiratory diseases.

(7a) The pharmaceutical preparation according to (6a), which is a prophylactic/therapeutic agent for respiratory diseases.

(8) An antibody against a protein comprising the same or substantially the same amino acid sequence as the amino acid sequence represented by SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 30, SEQ ID NO: 32, SEQ ID NO: 34, SEQ ID NO: 36, SEQ ID NO: 38, SEQ ID NO: 40, SEQ ID NO: 42, SEQ ID NO: 44, SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 58, SEQ ID NO: 60 or SEQ ID NO: 62, or against its partial peptide or a salt thereof.

(8a) An antibody against a protein comprising the same or substantially the same

amino acid sequence as the amino acid sequence represented by SEQ ID NO: 2, SEQ ID NO: 4 or SEQ ID NO: 30, or against its partial peptide or against a salt thereof.

(9) A pharmaceutical preparation comprising the antibody according to (8).

(9a) A pharmaceutical preparation comprising the antibody according to (8a).

(10) The pharmaceutical preparation according to (9), which is a prophylactic/therapeutic agent for respiratory diseases.

(10a) The pharmaceutical preparation according to (9a), which is a prophylactic/therapeutic agent for respiratory diseases.

(11) A diagnostic agent comprising the antibody according to (8).

(12) The diagnostic agent according to (11), which is a diagnostic agent for respiratory diseases.

(13) A diagnostic agent for respiratory diseases comprising a polynucleotide encoding a protein comprising the same or substantially the same amino acid sequence as the amino acid sequence represented by SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 30, SEQ ID NO: 32, SEQ ID NO: 34, SEQ ID NO: 36, SEQ ID NO: 38, SEQ ID NO: 40, SEQ ID NO: 42, SEQ ID NO: 44, SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 58, SEQ ID NO: 60, or SEQ ID NO: 62, or its partial peptide.

(14) A prophylactic/therapeutic agent for respiratory diseases, which comprises a compound or its salt having an action of inhibiting a cholesterol hydroxylation activity.

(15) A method of screening a prophylactic/therapeutic agent for respiratory diseases, which comprises using a protein comprising the same or substantially the same amino acid sequence as the amino acid sequence represented by SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 30, SEQ ID NO: 32, SEQ ID NO: 34, SEQ ID NO: 36, SEQ ID NO: 38, SEQ ID NO: 40, SEQ ID NO: 42, SEQ ID NO: 44, SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 58, SEQ ID NO: 60, or SEQ ID NO: 62, its partial peptide, or a salt thereof.

(15a) A method of screening a prophylactic/therapeutic agent for respiratory diseases, which comprises using a protein comprising the same or substantially the same amino acid sequence as the amino acid sequence represented by SEQ ID NO: 2, its

partial peptide, or a salt thereof.

(15b) A method of screening a prophylactic/therapeutic agent for cancer, which comprises using a protein comprising the same or substantially the same amino acid sequence as the amino acid sequence represented by SEQ ID NO: 4, its partial peptide, or a salt thereof.

(15c) A method of screening a prophylactic/therapeutic agent for cancer, which comprises using a protein comprising the same or substantially the same amino acid sequence as the amino acid sequence represented by SEQ ID NO: 30, its partial peptide, or a salt thereof.

(15d) A prophylactic/therapeutic agent for respiratory diseases, which is obtained by using the screening method according to (15) to (15c).

(16) The screening method according to (15), which comprises using a protein comprising the amino acid sequence represented by SEQ ID NO: 2, its partial peptide, or a salt thereof.

(17) A kit for screening a prophylactic/therapeutic agent for respiratory diseases, comprising a protein comprising the same or substantially the same amino acid sequence as the amino acid sequence represented by SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 30, SEQ ID NO: 32, SEQ ID NO: 34, SEQ ID NO: 36, SEQ ID NO: 38, SEQ ID NO: 40, SEQ ID NO: 42, SEQ ID NO: 44, SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 58, SEQ ID NO: 60, or SEQ ID NO: 62, its partial peptide, or a salt thereof.

(17a) A kit for screening a prophylactic/therapeutic agent for respiratory diseases, comprising a protein comprising the same or substantially the same amino acid sequence as the amino acid sequence represented by SEQ ID NO: 2, its partial peptide, or a salt thereof.

(17b) A kit for screening a prophylactic/therapeutic agent for respiratory diseases, comprising a protein comprising the same or substantially the same amino acid sequence as the amino acid sequence represented by SEQ ID NO: 4, its partial peptide, or a salt thereof.

(17c) A kit for screening a prophylactic/therapeutic agent for respiratory diseases, comprising a protein comprising the same or substantially the same amino acid sequence as the amino acid sequence represented by SEQ ID NO: 30, its partial peptide, or a salt thereof.

(17d) A prophylactic/therapeutic agent for respiratory diseases, which is obtainable by using the screening kit according to (17) to (17c).

(18) The screening kit according to (17), which comprises a protein comprising the amino acid sequence represented by SEQ ID NO: 2, its partial peptide, or a salt thereof.

(19) A method of screening a prophylactic/therapeutic agent for respiratory diseases, which comprises using a polynucleotide encoding a protein comprising the same or substantially the same amino acid sequence as the amino acid sequence represented by SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 30, SEQ ID NO: 32, SEQ ID NO: 34, SEQ ID NO: 36, SEQ ID NO: 38, SEQ ID NO: 40, SEQ ID NO: 42, SEQ ID NO: 44, SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 58, SEQ ID NO: 60, or SEQ ID NO: 62, or its partial peptide.

(19a) A method of screening a prophylactic/therapeutic agent for respiratory diseases, which comprises using a polynucleotide encoding a protein comprising the same or substantially the same amino acid sequence as the amino acid sequence represented by SEQ ID NO: 2, or its partial peptide.

(19b) A method of screening a prophylactic/therapeutic agent for respiratory diseases, which comprises using a polynucleotide encoding a protein comprising the same or substantially the same amino acid sequence as the amino acid sequence represented by SEQ ID NO: 4, or its partial peptide.

(19c) A method of screening a prophylactic/therapeutic agent for respiratory diseases, which comprises using a polynucleotide encoding a protein comprising the same or substantially the same amino acid sequence as the amino acid sequence represented by SEQ ID NO: 30, or its partial peptide.

(19d) A prophylactic/therapeutic agent for respiratory diseases, which is obtainable by using the screening method according to (19) to (19c).

(20) The screening method according to (19), which comprises using a polynucleotide encoding a protein comprising the amino acid sequence represented by SEQ ID NO: 2 or its partial peptide.

(21) A kit for screening a prophylactic/therapeutic agent for respiratory diseases, comprising a polynucleotide encoding a protein comprising the same or substantially the same amino acid sequence as the amino acid sequence represented by SEQ ID NO: 2,

SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 30, SEQ ID NO: 32, SEQ ID NO: 34, SEQ ID NO: 36, SEQ ID NO: 38, SEQ ID NO: 40, SEQ ID NO: 42, SEQ ID NO: 44, SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 58, SEQ ID NO: 60, or SEQ ID NO: 62, or its partial peptide.

(21a) A kit for screening a prophylactic/therapeutic agent for respiratory diseases, comprising a polynucleotide encoding a protein comprising the same or substantially the same amino acid sequence as the amino acid sequence represented by SEQ ID NO: 2, or its partial peptide.

(21b) A kit for screening a prophylactic/therapeutic agent for respiratory diseases, comprising the same or substantially the same amino acid sequence as the amino acid sequence represented by SEQ ID NO: 4.

(21c) A kit for screening a prophylactic/therapeutic agent for respiratory diseases, comprising a polynucleotide encoding a protein comprising the same or substantially the same amino acid sequence as the amino acid sequence represented by SEQ ID NO: 30, or its partial peptide.

(21d) A prophylactic/therapeutic agent for respiratory diseases, which is obtainable by using the screening kit according to (21) to (21c).

(22) The screening kit according to (21), comprising a polynucleotide encoding a protein comprising the amino acid sequence represented by SEQ ID NO: 2, or its partial peptide.

(23) A method of preventing/treating respiratory diseases, which comprises administering to a mammal an effective dose of a compound or its salt that inhibits the activity of a protein comprising the same or substantially the same amino acid sequence as the amino acid sequence represented by SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 30, SEQ ID NO: 32, SEQ ID NO: 34, SEQ ID NO: 36, SEQ ID NO: 38, SEQ ID NO: 40, SEQ ID NO: 42, SEQ ID NO: 44, SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 58, SEQ ID NO: 60 or SEQ ID NO: 62, or its partial peptide or a salt thereof, or a compound or its salt that inhibits the expression of a gene for the protein.

(24) The method according to (23), wherein the compound is a compound that

inhibits the activity of a protein comprising the amino acid sequence represented by SEQ ID NO: 2, its partial peptide, or a salt thereof, or a compound that inhibits the expression of a gene for the protein.

(25) A method of preventing/treating respiratory diseases, which comprises inhibiting the activity of a protein comprising the same or substantially the same amino acid sequence as the amino acid sequence represented by SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 30, SEQ ID NO: 32, SEQ ID NO: 34, SEQ ID NO: 36, SEQ ID NO: 38, SEQ ID NO: 40, SEQ ID NO: 42, SEQ ID NO: 44, SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 58, SEQ ID NO: 60, or SEQ ID NO: 62, its partial peptide, or a salt thereof, or inhibiting the expression of a gene for said protein.

(26) The method according to (25), wherein the activity of a protein comprising the amino acid sequence represented by SEQ ID NO: 2, its partial peptide, or a salt thereof, or the expression of a gene for the protein, is inhibited.

(27) Use of a compound or its salt that inhibits the activity of a protein comprising the same or substantially the same amino acid sequence as the amino acid sequence represented by SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 30, SEQ ID NO: 32, SEQ ID NO: 34, SEQ ID NO: 36, SEQ ID NO: 38, SEQ ID NO: 40, SEQ ID NO: 42, SEQ ID NO: 44, SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 58, SEQ ID NO: 60 or SEQ ID NO: 62, or its partial peptide or a salt thereof, or a compound or its salt that inhibits the expression of a gene for the protein, to manufacture a prophylactic/therapeutic agent for respiratory diseases.

(28) Use according to (27), wherein the compound is a compound that inhibits the activity of a protein comprising the amino acid sequence represented by SEQ ID NO: 2, its partial peptide, or a salt thereof, or a compound that inhibits the expression of a gene for the protein.

(29) A prophylactic/therapeutic agent for respiratory diseases, which comprises a compound or a salt thereof that promotes the activity of a protein comprising the same or substantially the same amino acid sequence as the amino acid sequence represented by SEQ ID NO: 64 or SEQ ID NO: 66, or its partial peptide or a salt thereof.

(30) A prophylactic/therapeutic agent for respiratory diseases, which comprises a compound or a salt thereof that promotes the expression of a gene for a protein comprising the same or substantially the same amino acid sequence represented by SEQ ID NO: 64 or SEQ ID NO: 66, or its partial peptide or a salt thereof.

(31) An antibody against a protein comprising the same or substantially the same amino acid sequence as the amino acid sequence represented by SEQ ID NO: 64 or SEQ ID NO: 66, or against its partial peptide or against a salt thereof.

(32) A diagnostic agent comprising the antibody according to (31).

(33) The diagnostic agent according to (34) [sic], which is a diagnostic agent for respiratory diseases.

(34) A diagnostic agent for respiratory diseases, comprising a polynucleotide encoding a protein comprising the same or substantially the same amino acid sequence as the amino acid sequence represented by SEQ ID NO: 64 or SEQ ID NO: 66, or its partial peptide.

(35) A method of screening a prophylactic/therapeutic agent for respiratory diseases, which comprises using a protein comprising the same or substantially the same amino acid sequence as the amino acid sequence represented by SEQ ID NO: 64 or SEQ ID NO: 66, its partial peptide, or a salt thereof.

(36) A kit for screening a prophylactic/therapeutic agent for respiratory diseases, comprising a protein comprising the same or substantially the same amino acid sequence as the amino acid sequence represented by SEQ ID NO: 64 or SEQ ID NO: 66, its partial peptide, or a salt thereof.

(37) A method of screening a prophylactic/therapeutic agent for respiratory diseases, which comprises using a polynucleotide encoding a protein comprising the same or substantially the same amino acid sequence as the amino acid sequence represented by SEQ ID NO: 64 or SEQ ID NO: 66, or its partial peptide.

(38) A kit for screening a prophylactic/therapeutic agent for respiratory diseases, comprising a polynucleotide encoding a protein comprising the same or substantially the same amino acid sequence as the amino acid sequence represented by SEQ ID NO: 64 or SEQ ID NO: 66, or its partial peptide.

(39) A method of preventing/treating respiratory diseases, which comprises administering to a mammal an effective dose of a compound or its salt that promotes the activity of a protein comprising the same or substantially the same amino acid sequence as the amino acid sequence represented by SEQ ID NO: 64 or SEQ ID NO: 66, or its partial peptide or a salt thereof, or a compound or its salt that promotes the expression of

a gene for the protein.

(40) A method of preventing/treating respiratory diseases, which comprises promoting the activity of a protein comprising the same or substantially the same amino acid sequence as the amino acid sequence represented by SEQ ID NO: 64 or SEQ ID NO: 66, or its partial peptide or a salt thereof, or promoting the expression of a gene for the protein.

(41) Use of a compound or its salt that promotes the activity of a protein comprising the same or substantially the same amino acid sequence as the amino acid sequence represented by SEQ ID NO: 64 or SEQ ID NO: 66, or its partial peptide or a salt thereof, or a compound or its salt that promotes the expression of a gene for the protein, to manufacture a prophylactic/therapeutic agent for respiratory diseases.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a graph showing the expression level of CH25H gene in each group.

Fig. 2 is a graph showing the correlation between the expression level of CH25H gene and forced expiratory volume in 1 second (%FEV1). In the graph, △ shows the NN group, □ shows the NE group, ○ shows the NS group, ▲ shows CE1 group, and ■ shows the CE2A group. The expression level of CH25H gene is shown in the ordinate and volume in 1 second (%FEV1) in the abscissa. r (correlation factor) = 0.36. p (statistically significant difference) = 1.9.

Fig. 3 is a graph showing the correlation between the expression level of CH25H gene and %Diffusing capacity of the lung for carbon monoxide (%DLCO). In the graph, △ shows the NN group, □ shows the NE group, ○ shows the NS group, ▲ shows CE1 group, and ■ shows the CE2A group. The expression level of CH25H gene is shown in the ordinate and %Diffusing capacity of the lung for carbon monoxide %DLCO in the abscissa. r (correlation factor) = – 0.81. p (statistically significant difference) = 0.0002.

Fig. 4(A) is a graph showing the expression levels of CH25H gene and CYP27A1 gene in the lung of a mouse exposed to cigarette smoke. In the graph, the expression level of each gene is shown in the ordinate and the duration of exposure to cigarette smoke in the abscissa.

Fig. 4(B) is a graph showing the expression levels of CH25H gene and CYP27A1 gene in cells in a bronchoalveolar lavage fluid from a mouse exposed to cigarette smoke. In the graph, the expression level of each gene is shown in the ordinate and the duration of exposure to cigarette smoke in the abscissa.

Fig. 5(A) is a graph showing a fluctuation in the amount of 25-hydroxycholesterol (25-HC) in the lung tissue of a mouse exposed to cigarette smoke. In the graph, the amount of 25-HC is shown in the ordinate, and the number of days on which the mouse was exposed to cigarette smoke is shown in the abscissa. • shows 25-HC, and ○ shows the control.

Fig. 5(B) is a graph showing a fluctuation in the amount of cholesterol in the lung tissue of a mouse exposed to cigarette smoke. In the graph, the amount of cholesterol is shown in the ordinate, and the number of days on which the mouse was exposed to cigarette smoke is shown in the abscissa. • shows 25-HC, and ○ shows the control.

Fig. 6(A) is a graph showing the expression level of CXCL2 gene upon stimulation, with LPS and 25-HC, of cells in a bronchoalveolar lavage fluid from a cigarette smoke-exposed mouse. In the graph, the expression level of each gene is shown in the ordinate and the amounts of LPS and 25-HC in the abscissa.

Fig. 6(B) is a graph showing the expression level of IL-1β gene upon stimulation, with LPS and 25-HC, of cells in a bronchoalveolar lavage fluid from a cigarette smoke-exposed mouse. In the graph, the expression level of each gene is shown in the ordinate and the amounts of LPS and 25-HC in the abscissa.

Fig. 7 is a graph showing the amount of cytokine in a bronchoalveolar lavage fluid after an intratracheal administration of 25-H. In the graph, the amount of cytokine is shown in the ordinate and the time after intratracheal administration is shown in the abscissa. • shows 25-HC, and ○ shows the control.

Fig. 8 is a graph showing the number of neutrophils in a bronchoalveolar lavage fluid after intratracheal administration of hydroxylated cholesterols. In the graph, the number of neutrophils is shown in the ordinate, and the intratracheally administered hydroxylated cholesterols are shown in the abscissa.

BEST MODE FOR CARRYING OUT THE INVENTION

The protein, which has the same or substantially the same amino acid sequence as the amino acid sequence represented by SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 30, SEQ ID NO: 32, SEQ ID NO: 34, SEQ ID NO: 36, SEQ ID NO: 38, SEQ ID NO: 40, SEQ ID NO: 42, SEQ ID NO: 44, SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 56,

SEQ ID NO: 58, SEQ ID NO: 60, SEQ ID NO: 62, SEQ ID NO: 64 or SEQ ID NO: 66 (hereinafter the protein is sometimes referred to as the protein of the present invention or the protein used in the present invention) may be any protein derived from any cells of human and warm-blooded animals (e.g., guinea pig, rat, mouse, fowl, rabbit, swine, ovine, bovine, simian, etc.) (such as hepatocytes, splenocytes, nerve cells, glial cells, β cells of pancreas, bone marrow cells, mesangial cells, Langerhans' cells, epidermic cells, epithelial cells, goblet cells, endothelial cells, smooth muscle cells, fibroblasts, fibrocytes, myocytes, fat cells, immune cells (e.g., macrophage, T cells, B cells, natural killer cells, mast cells, neutrophils, basophils, eosinophils, monocytes), megakaryocytes, synovial cells, chondrocytes, bone cells, osteoblasts, osteoclasts, mammary gland cells, hepatocytes or interstitial cells; or the corresponding precursor cells, stem cells, cancer cells, etc.); or any tissues where such cells are present, such as brain or any of brain regions (e.g., olfactory bulb, amygdaloid nucleus, basal ganglia, hippocampus, thalamus, hypothalamus, cerebral cortex, medulla oblongata, cerebellum), spinal cord, hypophysis, stomach, pancreas, kidney, liver, gonad, thyroid, gall-bladder, bone marrow, adrenal gland, skin, muscle, lung, gastrointestinal tract (e.g., large intestine and small intestine), blood vessel, heart, thymus, spleen, submandibular gland, peripheral blood, prostate, testis, ovary, placenta, uterus, bone, joint, skeletal muscle, etc.; the protein may also be a synthetic protein.

The amino acid sequence having substantially the same amino acid sequence as that represented by SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 30, SEQ ID NO: 32, SEQ ID NO: 34, SEQ ID NO: 36, SEQ ID NO: 38, SEQ ID NO: 40, SEQ ID NO: 42, SEQ ID NO: 44, SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 58, SEQ ID NO: 60, SEQ ID NO: 62, SEQ ID NO: 64 or SEQ ID NO: 66 includes amino acid sequences having at least about 50% homology, preferably at least about 60% homology, more preferably at least about 70% homology, much more preferably at least about 80% homology, further much more preferably at least about 90% homology and most preferably at least about 95% homology, to the amino acid sequence shown by SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 30, SEQ ID NO: 32, SEQ ID NO: 34, SEQ ID NO: 36, SEQ ID NO: 38, SEQ ID NO: 40, SEQ ID NO: 42,

SEQ ID NO: 44, SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 58, SEQ ID NO: 60, SEQ ID NO: 62, SEQ ID NO: 64 or SEQ ID NO: 66; and so on.

Homology of the amino acid sequences can be determined under the following conditions (an expectation value = 10; gaps are allowed; matrix = BLOSUM62; filtering = OFF) using a homology scoring algorithm NCBI BLAST (National Center for Biotechnology Information Basic Local Alignment Search Tool).

Preferred examples of the protein comprising substantially the same amino acid sequence as the amino acid sequence represented by SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 30, SEQ ID NO: 32, SEQ ID NO: 34, SEQ ID NO: 36, SEQ ID NO: 38, SEQ ID NO: 40, SEQ ID NO: 42, SEQ ID NO: 44, SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 58, SEQ ID NO: 60, SEQ ID NO: 62, SEQ ID NO: 64 or SEQ ID NO: 66 include proteins having substantially the same amino acid sequence as the amino acid sequence represented by SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 30, SEQ ID NO: 32, SEQ ID NO: 34, SEQ ID NO: 36, SEQ ID NO: 38, SEQ ID NO: 40, SEQ ID NO: 42, SEQ ID NO: 44, SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 58, SEQ ID NO: 60, SEQ ID NO: 62, SEQ ID NO: 64 or SEQ ID NO: 66 and having an activity of substantially the same nature as that of the protein comprising the amino acid sequence represented by SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 30, SEQ ID NO: 32, SEQ ID NO: 34, SEQ ID NO: 36, SEQ ID NO: 38, SEQ ID NO: 40, SEQ ID NO: 42, SEQ ID NO: 44, SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 58, SEQ ID NO: 60, SEQ ID NO: 62, SEQ ID NO: 64 or SEQ ID NO: 66, and the like.

The activity of substantially the same nature as that of the protein comprising the amino acid sequence represented by SEQ ID NO: 2 includes, for example, a cholesterol hydroxylation activity.

The activity of substantially the same nature as that of the protein comprising

the amino acid sequence represented by SEQ ID NO: 4 includes, for example, a neutrophil infiltration activity via interleukin 8 production from macrophage.

The activity of substantially the same nature as that of the protein comprising the amino acid sequence represented by SEQ ID NO: 30 includes, for example, a proteolysis enzyme activity.

The "substantially the same nature" is used to mean that the characteristics of these activities is equivalent in terms of its nature (e.g., physiologically or pharmacologically). Thus, the cholesterol hydroxylation activity, neutrophil infiltration activity and proteolysis enzyme activity described above are preferably equivalent (e.g., about 0.01 to 100 times, preferably about 0.1 to 10 times, more preferably 0.5 to 2 times), but differences in degree such as a level of these activities, quantitative factors such as a molecular weight of the protein may be present and allowable.

The cholesterol hydroxylation activity can be assayed by methods known per se, for example, a method described in J. Biol. Chem. 273:34316-34327 (1998) or with its modifications.

Specifically, the protein of the present invention (preferably a protein comprising the same or substantially the same amino acid sequence as the amino acid sequence represented by SEQ ID NO: 2) is reacted with a labeled cholesterol substrate, and the product is separated from the substrate by thin layer chromatography, and the amount of the product (for example, radioactivity) is measured to determine the cholesterol hydroxylation activity. As the labeled cholesterol substrate, cholesterol labeled with a radioisotope (for example, [^{125}I], [^{131}I], [^{3}H], [^{14}C] etc.) is used. Measurement of the radioactivity is carried out in a known method using a scintillation counter etc.

Measurement of the neutrophil infiltration activity can be carried out according to methods known per se, for example a method described in J. Immunol. 171:2057-2065 (2003) or with its modifications.

The protein of the present invention (preferably a protein comprising the same or substantially the same amino acid sequence as the amino acid sequence represented by SEQ ID NO: 4) and interleukin 8, together with a cell culture medium, are introduced into a lower chamber of Trans-well (manufactured by Corning), while neutrophils are added to an upper chamber, and the number of neutrophils passing through an endothelial cell layer and infiltrating from the upper chamber to the lower chamber is determined, whereby the neutrophil infiltration activity is measured.

The proteolysis enzyme activity can be assayed by methods known per se, for

example, a method described in J. Biol. Chem. 272:4281-4286 (1997) or with its modifications.

The protein of the present invention (preferably a protein comprising the same or substantially the same amino acid sequence as the amino acid sequence represented by SEQ ID NO: 30) is reacted with a labeled substrate peptide, and the amount of the substrate peptide decomposed (for example, fluorescence intensity) is measured, whereby the proteolysis activity is measured. As the labeled substrate peptide, use is made of, for example, a substrate peptide (for example, Nma-Pro-Lys-Pro-Leu-Ala-Nva-Trp-Lys (Dnp)-NH_2, Nma: N-methyl anthranilic acid etc.) labeled with a fluorescent substance (for example, fluorescamine, fluorescein isocyanate etc.). The fluorescence intensity is measured according to methods known in the art, for example a method using a fluorescence measuring apparatus.

Examples of the protein used in the present invention include so-called muteins such as proteins comprising (i) the amino acid sequence represented by SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 30, SEQ ID NO: 32, SEQ ID NO: 34, SEQ ID NO: 36, SEQ ID NO: 38, SEQ ID NO: 40, SEQ ID NO: 42, SEQ ID NO: 44, SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 58, SEQ ID NO: 60, SEQ ID NO: 62, SEQ ID NO: 64 or SEQ ID NO: 66, of which at least 1 or 2 (e.g., about 1 to about 100, preferably about 1 to about 30, more preferably about 1 to about 10 and most preferably several (1 to 5)) amino acids are deleted; (ii) the amino acid sequence represented by SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 30, SEQ ID NO: 32, SEQ ID NO: 34, SEQ ID NO: 36, SEQ ID NO: 38, SEQ ID NO: 40, SEQ ID NO: 42, SEQ ID NO: 44, SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 58, SEQ ID NO: 60, SEQ ID NO: 62, SEQ ID NO: 64 or SEQ ID NO: 66, to which at least 1 or 2 (e.g., about 1 to about 100, preferably about 1 to about 30, more preferably about 1 to about 10 and most preferably several (1 to 5)) amino acids are added; (iii) the amino acid sequence represented by SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 30, SEQ ID NO: 32,

SEQ ID NO: 34, SEQ ID NO: 36, SEQ ID NO: 38, SEQ ID NO: 40, SEQ ID NO: 42, SEQ ID NO: 44, SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 58, SEQ ID NO: 60, SEQ ID NO: 62, SEQ ID NO: 64 or SEQ ID NO: 66, in which at least 1 or 2 (e.g., about 1 to about 100, preferably about 1 to about 30, more preferably about 1 to about 10 and most preferably several (1 to 5)) amino acids are inserted; (iv) the amino acid sequence represented by SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 30, SEQ ID NO: 32, SEQ ID NO: 34, SEQ ID NO: 36, SEQ ID NO: 38, SEQ ID NO: 40, SEQ ID NO: 42, SEQ ID NO: 44, SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 58, SEQ ID NO: 60, SEQ ID NO: 62, SEQ ID NO: 64 or SEQ ID NO: 66, in which at least 1 or 2 (e.g., about 1 to about 100, preferably about 1 to about 30, more preferably about 1 to about 10 and most preferably several (1 to 5)) amino acids are substituted by other amino acids; or (v) a combination of these amino acid sequences; and the like.

Where the amino acid sequence contains insertion, deletion or substitution as described above, the position of its insertion, deletion or substitution is not particularly limited.

Throughout the specification, the proteins are represented in accordance with the conventional way of describing proteins, that is, the N-terminus (amino terminus) at the left hand and the C-terminus (carboxyl terminus) at the right hand. In the protein used in the present invention, the C-terminus may be in any form of a carboxyl group (-COOH), a carboxylate (-COO-), an amide ($-CONH_2$) and an ester (-COOR).

Herein, examples of the ester group shown by R include a C_{1-6} alkyl group such as methyl, ethyl, n-propyl, isopropyl, n-butyl, etc.; a C_{3-8} cycloalkyl group such as cyclopentyl, cyclohexyl, etc.; a C_{6-12} aryl group such as phenyl, α-naphthyl, etc.; a C_{7-14} aralkyl such as a phenyl-C_{1-2} alkyl group, e.g., benzyl, phenethyl, etc. or an α-naphthyl-C_{1-2} alkyl group such as α-naphthylmethyl, etc.; pivaloyloxymethyl and the like.

Where the protein used in the present invention contains a carboxyl group (or a carboxylate) at a position other than the C-terminus, the carboxyl group may be amidated or esterified and such an amide or ester is also included within the protein used in the present invention. Examples of the ester group in this case may be the C-terminal esters described above, etc.

Furthermore, examples of the protein used in the present invention include those in which the amino group at the N-terminal amino acid residues (e.g., methionine residue) is protected with a protecting group (e.g., a C_{1-6} acyl group such as a C_{1-6} alkanoyl group, e.g., formyl group, acetyl group, etc.); those wherein the N-terminal region is cleaved in vivo and the glutamyl group thus formed is pyroglutaminated; those wherein a substituent (e.g., -OH, -SH, amino group, imidazole group, indole group, guanidino group, etc.) on the side chain of an amino acid in the molecule is protected with a suitable protecting group (e.g., a C_{1-6} acyl group such as a C_{1-6} alkanoyl group, e.g., formyl group, acetyl group, etc.), or conjugated proteins such as glycoproteins having sugar chains; etc.

Specific examples of the protein used in the present invention include, for example, a protein comprising the amino acid sequence represented by SEQ ID NO: 2, a protein comprising the amino acid sequence represented by SEQ ID NO: 4, a protein comprising the amino acid sequence represented by SEQ ID NO: 6, a protein comprising the amino acid sequence represented by SEQ ID NO: 8, a protein comprising the amino acid sequence represented by SEQ ID NO: 10, a protein comprising the amino acid sequence represented by SEQ ID NO: 12, a protein comprising the amino acid sequence represented by SEQ ID NO: 14, a protein comprising the amino acid sequence represented by SEQ ID NO: 16, a protein comprising the amino acid sequence represented by SEQ ID NO: 18, a protein comprising the amino acid sequence represented by SEQ ID NO: 20, a protein comprising the amino acid sequence represented by SEQ ID NO: 22, a protein comprising the amino acid sequence represented by SEQ ID NO: 24, a protein comprising the amino acid sequence represented by SEQ ID NO: 26, a protein comprising the amino acid sequence represented by SEQ ID NO: 28, a protein comprising the amino acid sequence represented by SEQ ID NO: 30, a protein comprising the amino acid sequence represented by SEQ ID NO: 32, a protein comprising the amino acid sequence represented by SEQ ID NO: 34, a protein comprising the amino acid sequence represented by SEQ ID NO: 36, a protein comprising the amino acid sequence represented by SEQ ID NO: 38, a protein comprising the amino acid sequence represented by SEQ ID NO: 40, a protein comprising the amino acid sequence represented by SEQ ID NO: 42, a protein comprising the amino acid sequence represented by SEQ ID NO: 44, a protein comprising the amino acid sequence represented by SEQ ID NO: 46, a protein comprising the amino acid sequence represented by SEQ ID NO: 48, a protein comprising the amino acid sequence

represented by SEQ ID NO: 50, a protein comprising the amino acid sequence represented by SEQ ID NO: 52, a protein comprising the amino acid sequence represented by SEQ ID NO: 54, a protein comprising the amino acid sequence represented by SEQ ID NO: 56, a protein comprising the amino acid sequence represented by SEQ ID NO: 58, a protein comprising the amino acid sequence represented by SEQ ID NO: 60, a protein comprising the amino acid sequence represented by SEQ ID NO: 62, a protein comprising the amino acid sequence represented by SEQ ID NO: 64, a protein comprising the amino acid sequence represented by SEQ ID NO: 66, etc.

The partial peptide of the protein used in the present invention may be any peptide as long as it is a partial peptide of the protein used in the present invention described above and preferably has the property equivalent to that of the protein used in the present invention described above.

Specific examples include a peptide having an amino acid sequence in positions 1 to 272 in the amino acid sequence represented by SEQ ID NO: 2, a peptide having an amino acid sequence in positions 1 to 308 in the amino acid sequence represented by SEQ ID NO: 4, etc. Preferably used are peptides having, e.g., at least 20, preferably at least 50, more preferably at least 70, much more preferably at least 100, and most preferably at least 200, amino acids in the constituent amino acid sequence of the protein used in the present invention, and the like.

The partial peptide used in the present invention may contain deletion of at least 1 or 2 (preferably about 1 to about 10 and most preferably several (1 to 5)) amino acids in the amino acid sequence; addition of at least 1 or 2 (preferably about 1 to about 20, more preferably about 1 to about 10 and most preferably several (1 to 5)) amino acids in the amino acid sequence; insertion of at least 1 or 2 (preferably about 1 to about 20, more preferably about 1 to about 10 and most preferably several (1 to 5)) amino acids in the amino acid sequence; or substitution of at least 1 or 2 (preferably about 1 to about 10, more preferably about several amino acids and most preferably several (1 to 5)) amino acids in the amino acid sequence by other amino acids.

In the partial peptide used in the present invention, the C-terminus may be in any form of a carboxyl group (-COOH), a carboxylate (-COO-), an amide ($-CONH_2$) or an ester (-COOR).

Furthermore, the partial peptide used in the present invention includes those having a carboxyl group (or a carboxylate) at a position other than the C-terminus, those having an amino group protected with a protecting group at the N-terminal amino acid

residues (e.g., methionine residue); those being cleaved at the N-terminal region in vivo and with the glutamyl group thus formed being pyroglutaminated; those having a substituent on the side chain of an amino acid in the molecule wherein the substituent is protected with a suitable protecting group, or conjugated peptides such as so-called glycopeptides having sugar chains; etc., as in the protein used in the present invention described above.

The partial peptide used in the present invention may also be used as an antigen for producing antibodies.

As salts of the protein or partial peptide used in the present invention, salts with physiologically acceptable acids (e.g., inorganic acids or organic acids) or bases (e.g., alkali metal salts) may be employed, preferably in the form of physiologically acceptable acid addition salts. Examples of such salts include salts with inorganic acids (e.g., hydrochloric acid, phosphoric acid, hydrobromic acid, sulfuric acid), salts with organic acids (e.g., acetic acid, formic acid, propionic acid, fumaric acid, maleic acid, succinic acid, tartaric acid, citric acid, malic acid, oxalic acid, benzoic acid, methanesulfonic acid, benzenesulfonic acid) and the like.

The protein or partial peptide used in the present invention or salts thereof may be manufactured by publicly known methods used to purify a protein from human or warm-blooded animal cells or tissues described above. Alternatively, they may also be manufactured by culturing transformants containing DNAs encoding these proteins. Furthermore, they may also be manufactured by a modification of the methods for peptide synthesis, which will be later described.

Where these proteins are manufactured from human or mammalian tissues or cells, human or mammalian tissues or cells are homogenized, extracted with an acid or the like, and the extract is purified/isolated by a combination of chromatography techniques such as reverse phase chromatography, ion exchange chromatography, and the like.

To synthesize the protein or partial peptide used in the present invention or its salts, or amides thereof, commercially available resins that are used for protein synthesis may be used. Examples of such resins include chloromethyl resin, hydroxymethyl resin, benzhydrylamine resin, aminomethyl resin, 4-benzyloxybenzyl alcohol resin, 4-methylbenzhydrylamine resin, PAM resin, 4-hydroxymethylmethylphenyl acetamidomethyl resin, polyacrylamide resin, 4-(2', 4'-dimethoxyphenyl-hydroxymethyl)phenoxy resin, 4-(2', 4'-dimethoxyphenyl-Fmoc-aminoethyl) phenoxy resin, etc. Using these resins, amino

acids, in which α-amino groups and functional groups on the side chains are appropriately protected, are condensed on the resin in accordance with the sequence of the objective protein according to various condensation methods publicly known in the art. At the end of the reaction, the protein or partial peptide is excised from the resin and at the same time, the protecting groups are removed. Then, intramolecular disulfide bond-forming reaction is performed in a highly diluted solution to obtain the objective protein or partial peptide, or amides thereof.

For condensation of the protected amino acids described above, a variety of activation reagents for protein synthesis may be used, and carbodiimides are particularly employed. Examples of such carbodiimides include DCC, N, N' -diisopropylcarbodiimide, N-ethyl-N'-(3-dimethylaminopropyl) carbodiimide, etc. For activation by these reagents, the protected amino acids in combination with a racemization inhibitor (e.g., HOBt, HOOBt) are added directly to the resin, or the protected amino acids are previously activated in the form of symmetric acid anhydrides, HOBt esters or HOOBt esters, followed by adding the thus activated protected amino acids to the resin.

Solvents suitable for use to activate the protected amino acids or condense with the resin may be appropriately chosen from solvents that are known to be usable for protein condensation reactions. Examples of such solvents are acid amides such as N, N-dimethylformamide, N, N-dimethylacetamide, N-methylpyrrolidone, etc.; halogenated hydrocarbons such as methylene chloride, chloroform, etc.; alcohols such as trifluoroethanol, etc.; sulfoxides such as dimethylsulfoxide, etc.; ethers such as pyridine, dioxane, tetrahydrofuran, etc.; nitriles such as acetonitrile, propionitrile, etc.; esters such as methyl acetate, ethyl acetate, etc.; and appropriate mixtures of these solvents. The reaction temperature is appropriately chosen from the range known to be applicable to protein binding reactions and is usually selected in the range of approximately -20°C to 50°C. The activated amino acid derivatives are used generally in an excess of 1.5 to 4 times. The condensation is examined using the ninhydrin reaction; when the condensation is insufficient, the condensation can be completed by repeating the condensation reaction without removal of the protecting groups. When the condensation is yet insufficient even after repeating the reaction, unreacted amino acids are acetylated with acetic anhydride or acetylimidazole to avoid any possible effect on the subsequent reaction.

Examples of the protecting groups used to protect the starting amino groups include Z, Boc, t-pentyloxycarbonyl, isobornyloxycarbonyl,

4-methoxybenzyloxycarbonyl, Cl-Z, Br-Z, adamantyloxycarbonyl, trifluoroacetyl, phthaloyl, formyl, 2-nitrophenylsulphenyl, diphenylphosphinothioyl, Fmoc, etc.

A carboxyl group can be protected by, e.g., alkyl esterification (linear, branched or cyclic alkyl esterification of, e.g., methyl, ethyl, propyl, butyl, t-butyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl, 2-adamantyl, etc.), aralkyl esterification (e.g., benzyl ester, 4-nitrobenzyl ester, 4-methoxybenzyl ester, 4-chlorobenzyl ester, benzhydryl ester, etc.), phenacyl esterification, benzyloxycarbonyl hydrazidation, t-butoxycarbonyl hydrazidation, trityl hydrazidation, or the like.

The hydroxyl group of serine can be protected through, for example, its esterification or etherification. Examples of groups appropriately used for the esterification include a lower (C_{1-6}) alkanoyl group, such as acetyl group, an aroyl group such as benzoyl group, and a group derived from carbonic acid such as benzyloxycarbonyl group, ethoxycarbonyl group, etc. Examples of a group appropriately used for the etherification include benzyl group, tetrahydropyranyl group, t-butyl group, etc.

Examples of groups for protecting the phenolic hydroxyl group of tyrosine include Bzl, Cl2-Bzl, 2-nitrobenzyl, Br-Z, t-butyl, etc.

Examples of groups used to protect the imidazole moiety of histidine include Tos, 4-methoxy-2, 3, 6-trimethylbenzenesulfonyl, DNP, benzyloxymethyl, Bum, Boc, Trt, Fmoc, etc.

Examples of the activated carboxyl groups in the starting material include the corresponding acid anhydrides, azides, activated esters [esters with alcohols (e.g., pentachlorophenol, 2, 4, 5-trichlorophenol, 2, 4-dinitrophenol, cyanomethyl alcohol, p-nitrophenol, HONB, N-hydroxysuccimide, N-hydroxyphthalimide, HOBt)], etc. As the amino acids in which the amino groups are activated in the starting material, the corresponding phosphoric amides are employed.

To eliminate (split off) the protecting groups, there are used catalytic reduction under hydrogen gas flow in the presence of a catalyst such as Pd-black or Pd-carbon; an acid treatment with anhydrous hydrogen fluoride, methanesulfonic acid, trifluoromethanesulfonic acid, trifluoroacetic acid, or a mixture solution of these acids; a treatment with a base such as diisopropylethylamine, triethylamine, piperidine or piperazine; reduction with sodium in liquid ammonia, etc. The elimination of the protecting group by the acid treatment described above is carried out generally at a temperature of approximately -20°C to 40°C. In the acid treatment, it is efficient to add a cation scavenger such as anisole, phenol, thioanisole, m-cresol, p-cresol,

dimethylsulfide, 1, 4-butanedithiol, 1, 2-ethanedithiol, etc. Furthermore, 2, 4-dinitrophenyl group known as the protecting group for the imidazole of histidine is removed by a treatment with thiophenol. Formyl group used as the protecting group of the indole of tryptophan is eliminated by the aforesaid acid treatment in the presence of 1, 2-ethanedithiol, 1,4-butanedithiol, etc. as well as by a treatment with an alkali such as a dilute sodium hydroxide solution, dilute ammonia, etc.

Protection of functional groups that should not be involved in the reaction of the starting materials, protecting groups, elimination of the protecting groups and activation of functional groups involved in the reaction may be appropriately selected from publicly known groups and publicly known means.

In another method for obtaining the amides of the desired protein or partial peptide, for example, the α-carboxyl group of the carboxy terminal amino acid is first protected by amidation; the peptide (protein) chain is then extended from the amino group side to a desired length. Then, a protein or partial peptide, in which only the protecting group of the N-terminal α-amino group of the peptide chain has been eliminated, and a protein or partial peptide, in which only the protecting group of the C-terminal carboxyl group has been eliminated, are manufactured. The two proteins or peptides are condensed in a mixture of the solvents described above. The details of the condensation reaction are the same as described above. After the protected protein or peptide obtained by the condensation is purified, all the protecting groups are eliminated by the method described above to give the desired crude protein or peptide. This crude protein or peptide is purified by various known purification means. Lyophilization of the major fraction gives the amide of the desired protein or peptide.

To prepare the esterified protein or peptide, for example, the α-carboxyl group of the carboxy terminal amino acid is condensed with a desired alcohol to prepare the amino acid ester, which is followed by procedures similar to the preparation of the amidated protein or peptide above to give the desired esterified protein or peptide.

The partial peptide used in the present invention or salts thereof can be manufactured by publicly known methods for peptide synthesis, or by cleaving the protein used in the present invention with an appropriate peptidase. The methods for peptide synthesis include, for example, either solid phase synthesis or liquid phase synthesis may be used. That is, the partial peptide or amino acids that can construct the partial peptide used in the present invention are condensed with the remaining part. Where the product contains protecting groups, these protecting groups are removed to give the desired peptide. Publicly known methods for condensation and elimination of

the protecting groups are described in (i) to (v) below.

(i) M. Bodanszky & M.A. Ondetti: Peptide Synthesis, Interscience Publishers, New York (1966)

(ii) Schroeder & Luebke: The Peptide, Academic Press, New York (1965)

(iii) Nobuo Izumiya, et al.: Peptide Gosei-no-Kiso to Jikken (Basics and experiments of peptide synthesis), published by Maruzen Co. (1975)

(iv) Haruaki Yajima & Shunpei Sakakibara: Seikagaku Jikken Koza (Biochemical Experiment) 1, Tanpakushitsu no Kagaku (Chemistry of Proteins) IV, 205 (1977)

(v) Haruaki Yajima ed.: Zoku Iyakuhin no Kaihatsu (A sequel to Development of Pharmaceuticals), Vol. 14, Peptide Synthesis, published by Hirokawa Shoten

After completion of the reaction, the product may be purified and isolated by a combination of conventional purification methods such as solvent extraction, distillation, column chromatography, liquid chromatography and recrystallization to give the partial peptide used in the present invention. When the partial peptide obtained by the above methods is in a free form, the partial peptide can be converted into an appropriate salt by a publicly known method or its modification; when the partial peptide is obtained in a salt form, it can be converted into a free form or other different salt form by a publicly known method or its modification.

The polynucleotide encoding the protein used in the present invention may be any polynucleotide so long as it contains the base sequence encoding the protein used in the present invention described above. Preferably, the polynucleotide is a DNA. The DNA may also be any one of genomic DNA, genomic DNA library, cDNA derived from the cells or tissues described above, cDNA library derived from the cells or tissues described above and synthetic DNA.

The vector used for the library may be any of bacteriophage, plasmid, cosmid, phagemid and the like. In addition, the DNA can be amplified by reverse transcriptase polymerase chain reaction (hereinafter abbreviated as RT-PCR) with total RNA or mRNA fraction prepared from the above-described cells or tissues.

The DNA encoding the protein used in the present invention may be any one of, for example, a DNA comprising the base sequence represented by SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 37, SEQ ID NO: 39, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53,

SEQ ID NO: 55, SEQ ID NO: 57, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, or SEQ ID NO: 65, or a DNA comprising a base sequence hybridizable to the base sequence represented by SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 37, SEQ ID NO: 39, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 57, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, or SEQ ID NO: 65 under high stringent conditions and encoding a protein which has the properties of substantially the same nature as those of the protein having the amino acid sequence represented by SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 30, SEQ ID NO: 32, SEQ ID NO: 34, SEQ ID NO: 36, SEQ ID NO: 38, SEQ ID NO: 40, SEQ ID NO: 42, SEQ ID NO: 44, SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 58, SEQ ID NO: 60, SEQ ID NO: 62, SEQ ID NO: 64 or SEQ ID NO: 66 described above.

Specific examples of the DNA that is hybridizable to the base sequence represented by SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 37, SEQ ID NO: 39, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 57, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, or SEQ ID NO: 65 under high stringent conditions include DNAs comprising at least about 50% homology, preferably at least about 60% homology, more preferably at least about 70% homology, much more preferably at least about 80% homology, further much more preferably at least about 90% homology and most preferably at least about 95% homology, to the base sequence represented by SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 37, SEQ ID NO: 39, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51,

SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 57, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, or SEQ ID NO: 65; and the like.

Homology in the base sequence can be measured under the following conditions (an expectation value = 10; gaps are allowed; filtering = ON; match score = 1; mismatch score = -3) using the homology scoring algorithm NCBI BLAST (National Center for Biotechnology Information Basic Local Alignment Search Tool).

The hybridization can be carried out by publicly known methods or by modifications thereof, for example, by the method described in Molecular Cloning, 2nd (J. Sambrook et al., Cold Spring Harbor Lab. Press, 1989). A commercially available library can also be used according to the instructions of the attached manufacturer's protocol. The hybridization can be carried out preferably under high stringent conditions.

The high stringent conditions used herein are, for example, those in a sodium concentration at about 19 to 40 mM, preferably about 19 to 20 mM at a temperature of about 50 to 70°C, preferably about 60 to 65°C. In particular, hybridization conditions in a sodium concentration at about 19 mM at a temperature of about 65°C are most preferred.

More specifically, a DNA comprising the base sequence represented by SEQ ID NO: 1, etc. are used as the DNA encoding the protein comprising the amino acid sequence represented by SEQ ID NO: 2; a DNA comprising the base sequence represented by SEQ ID NO: 3, etc. are used as the DNA encoding the protein comprising the amino acid sequence represented by SEQ ID NO: 4; a DNA comprising the base sequence represented by SEQ ID NO: 5, etc. are used as the DNA encoding the protein comprising the amino acid sequence represented by SEQ ID NO: 6; a DNA comprising the base sequence represented by SEQ ID NO: 7, etc. are used as the DNA encoding the protein comprising the amino acid sequence represented by SEQ ID NO: 8; a DNA comprising the base sequence represented by SEQ ID NO: 9, etc. are used as the DNA encoding the protein comprising the amino acid sequence represented by SEQ ID NO: 10; a DNA comprising the base sequence represented by SEQ ID NO: 11, etc. are used as the DNA encoding the protein comprising the amino acid sequence represented by SEQ ID NO: 12; a DNA comprising the base sequence represented by SEQ ID NO: 13, etc. are used as the DNA encoding the protein comprising the amino acid sequence represented by SEQ ID NO: 14; a DNA comprising the base sequence represented by SEQ ID NO: 15, etc. are used as the DNA encoding the protein comprising the amino acid sequence represented by SEQ ID NO: 16; a DNA comprising the base sequence

represented by SEQ ID NO: 17, etc. are used as the DNA encoding the protein comprising the amino acid sequence represented by SEQ ID NO: 18; a DNA comprising the base sequence represented by SEQ ID NO: 19, etc. are used as the DNA encoding the protein comprising the amino acid sequence represented by SEQ ID NO: 20; a DNA comprising the base sequence represented by SEQ ID NO: 21, etc. are used as the DNA encoding the protein comprising the amino acid sequence represented by SEQ ID NO: 22; a DNA comprising the base sequence represented by SEQ ID NO: 23, etc. are used as the DNA encoding the protein comprising the amino acid sequence represented by SEQ ID NO: 24; a DNA comprising the base sequence represented by SEQ ID NO: 25, etc. are used as the DNA encoding the protein comprising the amino acid sequence represented by SEQ ID NO: 26; a DNA comprising the base sequence represented by SEQ ID NO: 27, etc. are used as the DNA encoding the protein comprising the amino acid sequence represented by SEQ ID NO: 28; a DNA comprising the base sequence represented by SEQ ID NO: 29, etc. are used as the DNA encoding the protein comprising the amino acid sequence represented by SEQ ID NO: 30; a DNA comprising the base sequence represented by SEQ ID NO: 31, etc. are used as the DNA encoding the protein comprising the amino acid sequence represented by SEQ ID NO: 32; a DNA comprising the base sequence represented by SEQ ID NO: 33, etc. are used as the DNA encoding the protein comprising the amino acid sequence represented by SEQ ID NO: 34; a DNA comprising the base sequence represented by SEQ ID NO: 35, etc. are used as the DNA encoding the protein comprising the amino acid sequence represented by SEQ ID NO: 36; a DNA comprising the base sequence represented by SEQ ID NO: 37, etc. are used as the DNA encoding the protein comprising the amino acid sequence represented by SEQ ID NO: 38; a DNA comprising the base sequence represented by SEQ ID NO: 39, etc. are used as the DNA encoding the protein comprising the amino acid sequence represented by SEQ ID NO: 40; a DNA comprising the base sequence represented by SEQ ID NO: 41, etc. are used as the DNA encoding the protein comprising the amino acid sequence represented by SEQ ID NO: 42; a DNA comprising the base sequence represented by SEQ ID NO: 43, etc. are used as the DNA encoding the protein comprising the amino acid sequence represented by SEQ ID NO: 44; a DNA comprising the base sequence represented by SEQ ID NO: 45, etc. are used as the DNA encoding the protein comprising the amino acid sequence represented by SEQ ID NO: 46; a DNA comprising the base sequence represented by SEQ ID NO: 47, etc. are used as the DNA encoding the protein comprising the amino acid sequence represented by SEQ ID NO: 48; a DNA comprising the base sequence represented by SEQ ID NO: 49, etc.

are used as the DNA encoding the protein comprising the amino acid sequence represented by SEQ ID NO: 50; a DNA comprising the base sequence represented by SEQ ID NO: 51, etc. are used as the DNA encoding the protein comprising the amino acid sequence represented by SEQ ID NO: 52; a DNA comprising the base sequence represented by SEQ ID NO: 53, etc. are used as the DNA encoding the protein comprising the amino acid sequence represented by SEQ ID NO: 54; a DNA comprising the base sequence represented by SEQ ID NO: 55, etc. are used as the DNA encoding the protein comprising the amino acid sequence represented by SEQ ID NO: 56; a DNA comprising the base sequence represented by SEQ ID NO: 57, etc. are used as the DNA encoding the protein comprising the amino acid sequence represented by SEQ ID NO: 58; a DNA comprising the base sequence represented by SEQ ID NO: 59, etc. are used as the DNA encoding the protein comprising the amino acid sequence represented by SEQ ID NO: 60; a DNA comprising the base sequence represented by SEQ ID NO: 61, etc. are used as the DNA encoding the protein comprising the amino acid sequence represented by SEQ ID NO: 62; a DNA comprising the base sequence represented by SEQ ID NO: 63, etc. are used as the DNA encoding the protein comprising the amino acid sequence represented by SEQ ID NO: 64; and a DNA comprising the base sequence represented by SEQ ID NO: 65, etc. are used as the DNA encoding the protein comprising the amino acid sequence represented by SEQ ID NO: 66.

The polynucleotide (eg. the DNA) encoding the partial peptide used in the present invention may be any DNA so long as it contains the base sequence encoding the partial peptide used in the present invention described above. The DNA may also be any of genomic DNA, genomic DNA library, cDNA derived from the cells and tissues described above, cDNA library derived from the cells and tissues described above and synthetic DNA.

As the DNA encoding the partial peptide used in the present invention, there are employed, for example, (i) a DNA comprising a part of the DNA having the base sequence represented by SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 37, SEQ ID NO: 39, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 57, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, or SEQ ID NO: 65, or (ii) a DNA comprising a base sequence hybridizable to the base sequence represented by SEQ ID

NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 37, SEQ ID NO: 39, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 57, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, or SEQ ID NO: 65 under high stringent conditions and comprising a part of DNA encoding a protein having the activities of substantially the same nature as those of the protein of the present invention, and the like.

The DNA hybridizable to the base sequence represented by SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 37, SEQ ID NO: 39, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 57, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, or SEQ ID NO: 65 indicates the same meaning as described above.

Methods for the hybridization and the high stringent conditions that can be used are the same as those described above.

For cloning of the DNA that completely encodes the protein or partial peptide used in the present invention (hereinafter sometimes merely referred to as the protein of the present invention in the description of cloning of DNAs encoding the protein and partial peptide and their expression), the DNA can be either amplified by PCR using synthetic DNA primers containing a part of the base sequence of the protein of the present invention, or the DNA inserted into an appropriate vector can be selected by hybridization with a labeled DNA fragment or synthetic DNA that encodes a part or entire region of the protein of the present invention. The hybridization can be carried out, for example, according to the method described in Molecular Cloning, 2nd (J. Sambrook et al., Cold Spring Harbor Lab. Press, 1989). Where the hybridization is carried out using commercially available library, the procedures may be conducted in accordance with the protocol described in the attached instructions.

Substitution of the base sequence of DNA can be effected by publicly known methods such as the ODA-LA PCR method, the Gapped duplex method, the Kunkel method, etc., or its modification, using PCR, a publicly known kit available as MutanTM-super Express Km (manufactured by Takara Shuzo Co., Ltd.) or MutanTM-K

(manufactured by Takara Shuzo Co., Ltd.), etc.

The cloned DNA encoding the protein can be used as it is, depending upon purpose or, if desired, after digestion with a restriction enzyme or after addition of a linker thereto. The DNA may contain ATG as a translation initiation codon at the 5' end thereof and TAA, TGA or TAG as a translation termination codon at the 3' end thereof. These translation initiation and termination codons may also be added by using an appropriate synthetic DNA adapter.

The expression vector for the protein of the present invention can be manufactured, for example, by (a) excising the desired DNA fragment from the DNA encoding the protein of the present invention, and then (b) ligating the DNA fragment with an appropriate expression vector downstream a promoter in the vector.

Examples of the vector include plasmids derived form Escherichia coli (e.g., pBR322, pBR325, pUC12, pUC13), plasmids derived from Bacillus subtilis (e.g., pUB110, pTP5, pC194), plasmids derived from yeast (e.g., pSH19, pSH15), bacteriophages such as λ phage, etc., animal viruses such as retrovirus, vaccinia virus, baculovirus, etc. as well as pA1-11, pXT1, pRc/CMV, pRc/RSV, pcDNA I/Neo, etc.

The promoter used in the present invention may be any promoter if it matches well with a host to be used for gene expression. In the case of using animal cells as the host, examples of the promoter include SRα promoter, SV40 promoter, LTR promoter, CMV promoter, HSV-TK promoter, etc.

Among them, it is preferred to use CMV (cytomegalovirus) promoter, SRα promoter, etc. Where the host is bacteria of the genus Escherichia, preferred examples of the promoter include trp promoter, lac promoter, recA promoter, λPL promoter, lpp promoter, T7 promoter, etc. In the case of using bacteria of the genus Bacillus as the host, preferred example of the promoter are SPO1 promoter, SPO2 promoter, penP promoter, etc. When yeast is used as the host, preferred examples of the promoter are PHO5 promoter, PGK promoter, GAP promoter, ADH promoter, etc. When insect cells are used as the host, preferred examples of the promoter include polyhedrin promoter, P10 promoter, etc.

In addition to the foregoing examples, the expression vector may further optionally contain an enhancer, a splicing signal, a poly A addition signal, a selection marker, SV40 replication origin (hereinafter sometimes abbreviated as SV40ori), etc. Examples of the selection marker include dihydrofolate reductase (hereinafter sometimes abbreviated as dhfr) gene [methotrexate (MTX) resistance], ampicillin resistant gene (hereinafter sometimes abbreviated as Ampr), neomycin resistant gene (hereinafter

sometimes abbreviated as Neor, G418 resistance), etc. In particular, when dhfr gene is used as the selection marker using dhfr gene-deficient Chinese hamster cells, selection can also be made on a thymidine free medium.

If necessary, a signal sequence that matches with a host is added to the N-terminus of the protein of the present invention. Examples of the signal sequence that can be used are PhoA signal sequence, OmpA signal sequence, etc. when bacteria of the genus Escherichia is used as the host; α-amylase signal sequence, subtilisin signal sequence, etc. when bacteria of the genus Bacillus is used as the host; MFα signal sequence, SUC2 signal sequence, etc. when yeast is used as the host; and insulin signal sequence, α-interferon signal sequence, antibody molecule signal sequence, etc. when animal cells are used as the host, respectively.

Using the vector containing the DNA encoding the protein of the present invention thus constructed, transformants can be manufactured.

Examples of the host, which may be employed, are genus Escherichia, genus Bacillus, yeast, insect cells, insects, animal cells, etc.

Specific examples of the genus Escherichia include Escherichia coli K12 DH1 [Proc. Natl. Acad. Sci. U.S.A., 60, 160 (1968)], JM103 [Nucleic Acids Research, 9, 309 (1981)], JA221 [Journal of Molecular Biology, 120, 517 (1978)], HB101 [Journal of Molecular Biology, 41, 459 (1969)], C600 [Genetics, 39, 440 (1954)], etc.

Examples of the genus Bacillus include Bacillus subtilis MI114 [Gene, 24, 255 (1983)], 207-21 [Journal of Biochemistry, 95, 87 (1984)], etc.

Examples of yeast include Saccharomyces cerevisiae AH22, AH22R-, NA87-11A, DKD-5D, 20B-12, Schizosaccharomyces pombe NCYC1913, NCYC2036, Pichia pastoris KM71, etc.

Examples of insect cells include, for the virus AcNPV, Spodoptera frugiperda cell (Sf cell), MG1 cell derived from mid-intestine of Trichoplusia ni, High FiveTM cell derived from egg of Trichoplusia ni, cells derived from Mamestra brassicae, cells derived from Estigmena acrea, etc.; and for the virus BmNPV, Bombyx mori N cell (BmN cell), etc. is used. Examples of the Sf cell which can be used are Sf9 cell (ATCC CRL1711), Sf21 cell (both cells are described in Vaughn, J. L. et al., In Vivo, 13, 213-217 (1977)), etc.

As the insect, for example, a larva of Bombyx mori can be used [Maeda et al., Nature, 315, 592 (1985)].

Examples of animal cells include monkey cell COS-7, Vero, Chinese hamster cell CHO (hereinafter referred to as CHO cell), dhfr gene-deficient Chinese hamster cell

CHO (hereinafter simply referred to as CHO (dhfr-) cell), mouse L cell, mouse AtT-20, mouse myeloma cell, mouse ATDC5 cell, rat GH3, human FL cell, etc.

Bacteria belonging to the genus Escherichia can be transformed, for example, by the method described in Proc. Natl. Acad. Sci. U.S.A., 69, 2110 (1972), Gene, 17, 107 (1982), etc.

Bacteria belonging to the genus Bacillus can be transformed, for example, by the method described in Molecular & General Genetics, 168, 111 (1979), etc.

Yeast can be transformed, for example, by the method described in Methods in Enzymology, 194, 182-187 (1991), Proc. Natl. Acad. Sci. U.S.A., 75, 1929 (1978), etc.

Insect cells or insects can be transformed, for example, according to the method described in Bio/Technology, 6, 47-55 (1988), etc.

Animal cells can be transformed, for example, according to the method described in Saibo Kogaku (Cell Engineering), extra issue 8, Shin Saibo Kogaku Jikken Protocol (New Cell Engineering Experimental Protocol), 263-267 (1995) (published by Shujunsha), or Virology, 52, 456 (1973).

Thus, the transformants transformed with the expression vectors containing the DNAs encoding the protein can be obtained.

Where the host is bacteria belonging to the genus Escherichia or the genus Bacillus, the transformant can be appropriately cultured in a liquid medium, which contains materials required for growth of the transformant such as carbon sources, nitrogen sources, inorganic materials, and the like. Examples of the carbon sources include glucose, dextrin, soluble starch, sucrose, etc.; examples of the nitrogen sources include inorganic or organic materials such as ammonium salts, nitrate salts, corn steep liquor, peptone, casein, meat extract, soybean cake, potato extract, etc.; and, examples of the inorganic materials are calcium chloride, sodium dihydrogenphosphate, magnesium chloride, etc. In addition, yeast extracts, vitamins, growth promoting factors etc. may also be added to the medium. Preferably, pH of the medium is adjusted to about 5 to 8.

A preferred example of the medium for culturing the bacteria belonging to the genus Escherichia is M9 medium supplemented with glucose and Casamino acids [Miller, Journal of Experiments in Molecular Genetics, 431-433, Cold Spring Harbor Laboratory, New York, 1972]. If necessary, a chemical such as 3β-indolylacrylic acid can be added to the medium thereby to activate the promoter efficiently.

Where the bacteria belonging to the genus Escherichia are used as the host, the transformant is usually cultivated at about 15 to 43°C for about 3 to 24 hours. If necessary, the culture may be aerated or agitated.

Where the bacteria belonging to the genus Bacillus are used as the host, the transformant is cultured generally at about 30 to 40°C for about 6 to 24 hours. If necessary, the culture can be aerated or agitated.

Where yeast is used as the host, the transformant is cultivated, for example, in Burkholder's minimal medium [Bostian, K. L. et al., Proc. Natl. Acad. Sci. U.S.A., 77, 4505 (1980)] or in SD medium supplemented with 0.5% Casamino acids [Bitter, G. A. et al., Proc. Natl. Acad. Sci. U.S.A., 81, 5330 (1984)]. Preferably, pH of the medium is adjusted to about 5 to 8. In general, the transformant is cultivated at about 20 to 35°C for about 24 to 72 hours. If necessary, the culture can be aerated or agitated.

Where insect cells or insects are used as the host, the transformant is cultivated in, for example, Grace's Insect Medium (Grace, T. C. C., Nature), 195, 788 (1962)) to which an appropriate additive such as immobilized 10% bovine serum is added. Preferably, pH of the medium is adjusted to about 6.2 to 6.4. Normally, the transformant is cultivated at about 27°C for about 3 to 5 days and, if necessary, the culture can be aerated or agitated.

Where animal cells are employed as the host, the transformant is cultured in, for example, MEM medium containing about 5 to 20% fetal bovine serum [Science, 122, 501 (1952)], DMEM medium [Virology, 8, 396 (1959)], RPMI 1640 medium [The Journal of the American Medical Association, 199, 519 (1967)], 199 medium [Proceeding of the Society for the Biological Medicine, 73, 1 (1950)], etc. Preferably, pH of the medium is adjusted to about 6 to about 8. The transformant is usually cultivated at about 30 to 40°C for about 15 to 60 hours and, if necessary, the culture can be aerated or agitated.

As described above, the protein of the present invention can be produced in the cell of, in the cell membrane of, or outside of the transformant.

The protein of the present invention can be separated and purified from the culture described above by the following procedures.

When the protein of the present invention is extracted from the culture of bacteria or cells, the bacteria or cell is collected after culturing by a publicly known method and suspended in an appropriate buffer. The bacteria or cell is then disrupted by publicly known methods such as ultrasonication, a treatment with lysozyme and/or freeze-thaw cycling; followed by centrifugation, filtration, etc to produce crude extract of the protein. Thus, the crude extract of the protein can be obtained. The buffer used for the procedures may contain a protein modifier such as urea or guanidine hydrochloride, or a surfactant such as Triton X-100TM, etc. When the protein of the present invention

is secreted in the culture broth, the supernatant can be separated, after completion of the cultivation, from the bacteria or cell to collect the supernatant by a publicly known method.

The protein contained in the supernatant or the extract thus obtained can be purified by appropriately combining the publicly known methods for separation and purification. Such publicly known methods for separation and purification include a method utilizing difference in solubility such as salting out, solvent precipitation, etc.; a method mainly utilizing difference in molecular weight such as dialysis, ultrafiltration, gel filtration, SDS-polyacrylamide gel electrophoresis, etc.; a method utilizing difference in electric charge such as ion exchange chromatography, etc.; a method utilizing difference in specific affinity such as affinity chromatography, etc.; a method utilizing difference in hydrophobicity such as reverse phase high performance liquid chromatography, etc.; a method utilizing difference in isoelectric point such as isoelectrofocusing electrophoresis; and the like.

When the protein thus obtained is in a free form, the protein can be converted into the salt by publicly known methods or modifications thereof. On the other hand, when the protein is obtained in the form of a salt, it can be converted into the free form or in the form of a different salt by publicly known methods or modifications thereof.

The protein produced by the recombinant can be treated, prior to or after the purification, with an appropriate protein-modifying enzyme so that the protein can be subjected to addition of an appropriate modification or removal of a partial polypeptide. Examples of the protein-modifying enzyme include trypsin, chymotrypsin, arginyl endopeptidase, protein kinase, glycosidase and the like.

The presence of the thus produced protein of the present invention can be determined by an enzyme immunoassay or western blotting using a specific antibody.

The antibodies against the protein or partial peptide used in the present invention, or its salts may be any of polyclonal and monoclonal antibodies, as long as they are capable of recognizing the protein or partial peptide used in the present invention, or its salts.

The antibodies against the protein or partial peptide used in the present invention, or its salts (hereinafter they are sometimes collectively referred to as the protein of the present invention in the description of the antibodies) can be produced by a publicly known method of producing an antibody or antiserum, using the protein of the present invention as an antigen.

[Preparation of monoclonal antibody]

(a) Preparation of monoclonal antibody-producing cells

The protein of the present invention is administered to warm-blooded animals either solely or together with carriers or diluents to the site where the production of antibody is possible by the administration. In order to potentiate the antibody productivity upon the administration, complete Freund's adjuvants or incomplete Freund's adjuvants may be administered. The administration is usually carried out once every about 2 to about 6 weeks and about 2 to about 10 times in total. Examples of the applicable warm-blooded animals are simian, rabbits, canine, guinea pigs, mice, rats, ovine, goats and fowl, with the use of mice and rats being preferred.

In the preparation of monoclonal antibody-producing cells, a warm-blooded animal, e.g., mouse, immunized with an antigen wherein the antibody titer is noted is selected, then spleen or lymph node is collected after 2 to 5 days from the final immunization and antibody-producing cells contained therein are fused with myeloma cells from homozoic or heterozoic animal to give monoclonal antibody-producing hybridomas. Measurement of the antibody titer in antisera may be carried out, for example, by reacting a labeled protein, which will be described later, with the antiserum followed by assaying the binding activity of the labeling agent bound to the antibody. The fusion may be carried out, for example, by the known method by Koehler and Milstein [Nature, 256, 495, (1975)]. Examples of the fusion accelerator are polyethylene glycol (PEG), Sendai virus, etc., of which PEG is preferably employed.

Examples of the myeloma cells are those collected from warm-blooded animals such as NS-1, P3U1, SP2/0, AP-1, etc. In particular, P3U1 is preferably employed. A preferred ratio of the count of the antibody-producing cells used (spleen cells) to the count of myeloma cells is within a range of approximately 1:1 to 20:1. When PEG (preferably, PEG 1000 to PEG 6000) is added in a concentration of approximately 10 to 80% followed by incubation at 20 to 40°C, preferably at 30 to 37°C for 1 to 10 minutes, an efficient cell fusion can be carried out.

Various methods can be used for screening of monoclonal antibody-producing hybridomas. Examples of such methods include a method which comprises adding the supernatant of a hybridoma to a solid phase (e.g., a microplate) adsorbed with the protein as an antigen directly or together with a carrier, adding an anti-immunoglobulin antibody (where mouse cells are used for the cell fusion, anti-mouse immunoglobulin antibody is used) labeled with a radioactive substance or an enzyme or Protein A and detecting the monoclonal antibody bound to the solid phase, and a method which comprises adding the

supernatant of hybridoma to a solid phase adsorbed with an anti-immunoglobulin antibody or Protein A, adding the protein labeled with a radioactive substance or an enzyme and detecting the monoclonal antibody bound to the solid phase, or the like.

The monoclonal antibody can be screened according to publicly known methods or their modifications. In general, the screening can be performed in a medium for animal cells supplemented with HAT (hypoxanthine, aminopterin and thymidine). Any screening and growth medium can be employed as far as the hybridoma can grow there. For example, RPMI 1640 medium containing 1 to 20%, preferably 10 to 20% fetal bovine serum, GIT medium (Wako Pure Chemical Industries, Ltd.) containing 1 to 10% fetal bovine serum, a serum free medium for cultivation of a hybridoma (SFM-101, Nissui Seiyaku Co., Ltd.) and the like, can be used for the screening and growth medium. The culture is carried out generally at 20 to 40°C, preferably at 37°C, for about 5 days to about 3 weeks, preferably 1 to 2 weeks, normally under 5% CO_2. The antibody titer of the culture supernatant of a hybridoma can be determined as in the assay for the antibody titer in antisera described above.

(b) Purification of monoclonal antibody

Separation and purification of a monoclonal antibody can be carried out by publicly known methods, such as separation and purification of immunoglobulins [for example, salting-out, alcohol precipitation, isoelectric point precipitation, electrophoresis, adsorption and desorption with ion exchangers (e.g., DEAE), ultracentrifugation, gel filtration, or a specific purification method which comprises collecting only an antibody with an activated adsorbent such as an antigen-binding solid phase, Protein A or Protein G and dissociating the binding to obtain the antibody.]

[Preparation of polyclonal antibody]

The polyclonal antibody of the present invention can be manufactured by publicly known methods or modifications thereof. For example, a warm-blooded animal is immunized with an immunogen (protein antigen) per se, or a complex of immunogen and a carrier protein is formed and the animal is immunized with the complex in a manner similar to the method described above for the manufacture of monoclonal antibodies. The product containing the antibody against the protein of the present invention is collected from the immunized animal followed by separation and purification of the antibody.

In the complex of immunogen and carrier protein used to immunize a warm-blooded animal, the type of carrier protein and the mixing ratio of carrier to hapten

may be any type and in any ratio, as long as the antibody is efficiently produced to the hapten immunized by crosslinking to the carrier. For example, bovine serum albumin, bovine thyroglobulin or hemocyanin is coupled to hapten in a carrier-to-hapten weight ratio of approximately 0.1 to 20, preferably about 1 to 5.

A variety of condensation agents can be used for the coupling of carrier to hapten. Glutaraldehyde, carbodiimide, maleimide activated ester and activated ester reagents containing thiol group or dithiopyridyl group are used for the coupling.

The condensation product is administered to warm-blooded animals either solely or together with carriers or diluents to the site that can produce the antibody by the administration. In order to potentiate the antibody productivity upon the administration, complete Freund's adjuvant or incomplete Freund's adjuvant may be administered. The administration is usually made once every about 2 to 6 weeks and about 3 to 10 times in total.

The polyclonal antibody can be collected from the blood, ascites, etc., preferably from the blood of warm-blooded animal immunized by the method described above.

The polyclonal antibody titer in antiserum can be assayed by the same procedure as that for the determination of serum antibody titer described above. The separation and purification of the polyclonal antibody can be carried out, following the method for the separation and purification of immunoglobulins performed as in the separation and purification of monoclonal antibodies described above.

The antisense polynucleotide having a complementary or substantially complementary base sequence to the base sequence of a polynucleotide encoding the protein or partial peptide used in the present invention (e.g., DNA (hereinafter these DNAs are sometimes collectively referred to as the DNA of the present invention in the description of antisense polynucleotide)) can be any antisense polynucleotide, so long as it possesses a base sequence complementary or substantially complementary to the base sequence of the DNA of the present invention and capable of suppressing the expression of said DNA, but antisense DNA is preferred.

The base sequence substantially complementary to the DNA of the present invention may include, for example, a base sequence having at least about 70% homology, preferably at least about 80% homology, more preferably at least about 90% homology and most preferably at least about 95% homology, to the entire base sequence or to its partial base sequence (i.e., complementary strand to the DNA of the present invention), and the like. Especially in the entire base sequence of the complementary strand to the DNA of the present invention, preferred are (a) an antisense polynucleotide

having at least about 70% homology, preferably at least about 80% homology, more preferably at least about 90% homology and most preferably at least about 95% homology, to the complementary strand of the base sequence which encodes the N-terminal region of the protein of the present invention (e.g., the base sequence around the initiation codon) in the case of antisense polynucleotide directed to translation inhibition and (b) an antisense polynucleotide having at least about 70% homology, preferably at least about 80% homology, more preferably at least about 90% homology and most preferably at least about 95% homology, to the complementary strand of the entire base sequence of the DNA of the present invention having intron, in the case of antisense polynucleotide directed to RNA degradation by RNaseH, respectively.

Specific examples include an antisense polynucleotide containing the entire or part of a base sequence complementary or substantially complementary to a base sequence of DNA containing the base sequence represented by SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 37, SEQ ID NO: 39, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 57, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, or SEQ ID NO: 65, preferably an antisense polynucleotide containing the entire or part of a base sequence complementary to a base sequence of DNA containing the base sequence represented by SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 37, SEQ ID NO: 39, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 57, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, or SEQ ID NO: 65 (more preferably, an antisense polynucleotide containing the entire or part of a base sequence complementary to a base sequence of DNA containing the base sequence represented by SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 37, SEQ ID NO: 39, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53,

SEQ ID NO: 55, SEQ ID NO: 57, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, or SEQ ID NO: 65, etc.

The antisense polynucleotide is generally constituted by bases of about 10 to about 40, preferably about 15 to about 30.

To prevent digestion with a hydrolase such as nuclease, etc., the phosphoric acid residue (phosphate) of each nucleotide that constitutes the antisense DNA may be substituted with chemically modified phosphoric acid residues, e.g., phosphorothioate, methyl phosphonate, phosphorodithionate, etc. Also, the sugar (deoxyribose) in each nucleotide may be replaced by a chemically modified structure such as 2'-O-methylation, etc. The base part (pyrimidine, purine) may also be chemically modified and may be any one which hybridizes to a DNA containing the base sequence represented by SEQ ID NO: 2. These antisense polynucleotides may be synthesized using a publicly known DNA synthesizer, etc.

According to the present invention, the antisense polynucleotide capable of inhibiting the replication or expression of a gene for the protein of the present invention can be designed and synthesized based on the base sequence information of cloned or identified protein-encoding DNA. Such a nucleotide (a nucleic acid) is hybridizable to RNA of a gene for the protein of the present invention to inhibit the synthesis or function of said RNA or is capable of modulating and/or controlling the expression of a gene for the protein of the present invention via interaction with RNA associated with the protein of the present invention. Polynucleotides complementary to the selected sequences of RNA associated with the protein of the present invention and polynucleotides specifically hybridizable to RNA associated with the protein of the present invention are useful in modulating and/or controlling the in vivo and in vitro expression of the protein gene of the present invention, and are useful for the treatment or diagnosis of diseases, etc. The term "corresponding" is used to mean homologous to or complementary to a particular sequence of the nucleotide including the gene, base sequence or nucleic acid. The term "corresponding" between nucleotides, base sequences or nucleic acids and proteins usually refer to amino acids of a protein under the order derived from the sequence of nucleotides (nucleic acids) or their complements. In the protein genes, the 5' end hairpin loop, 5' end 6-base-pair repeats, 5' end untranslated region, polypeptide translation initiation codon, protein coding region, ORF translation termination codon, 3' end untranslated region, 3' end palindrome region, and 3' end hairpin loop, may be selected as preferred target regions, though any other region may be selected as a target in the protein genes.

The relationship between the targeted nucleic acids and the polynucleotides complementary to at least a part of the target, or the relationship between the target and the polynucleotides hybridizable with the target, can be denoted to be "antisense". Examples of the antisense polynucleotides include polynucleotides containing 2-deoxy-D-ribose, polynucleotides containing D-ribose, any other type of polynucleotides which are N-glycosides of a purine or pyrimidine base, or other polymers having non-nucleotide backbones (e.g., commercially available protein nucleic acids and synthetic sequence-specific nucleic acid polymers) or other polymers containing particular linkages (provided that the polymers contain nucleotides having such a configuration that allows base pairing or base stacking, as is found in DNA or RNA), etc. The antisense polynucleotides may be double-stranded DNA, single-stranded DNA, double-stranded RNA, single-stranded RNA or a DNA:RNA hybrid, and may further include unmodified polynucleotides (or unmodified oligonucleotides), those with publicly known types of modifications, for example, those with labels known in the art, those with caps, methylated polynucleotides, those with substitution of one or more naturally occurring nucleotides by their analogue, those with intramolecular modifications of nucleotides such as those with uncharged linkages (e.g., methyl phosphonates, phosphotriesters, phosphoramidates, carbamates, etc.) and those with charged linkages or sulfur-containing linkages (e.g., phosphorothioates, phosphorodithioates, etc.), those having side chain groups such as proteins (nucleases, nuclease inhibitors, toxins, antibodies, signal peptides, poly-L-lysine, etc.), saccharides (e.g., monosaccharides, etc.), those with intercalators (e.g., acridine, psoralen, etc.), those containing chelators (e.g., metals, radioactive metals, boron, oxidative metals, etc.), those containing alkylating agents, those with modified linkages (e.g., α anomeric nucleic acids, etc.), and the like. Herein the terms "nucleoside", "nucleotide" and "nucleic acid" are used to refer to moieties that contain not only the purine and pyrimidine bases, but also other heterocyclic bases, which have been modified. Such modifications may include methylated purines and pyrimidines, acylated purines and pyrimidines and other heterocyclic rings. Modified nucleotides and modified nucleotides also include modifications on the sugar moiety, wherein, for example, one or more hydroxyl groups may optionally be substituted with a halogen atom(s), an aliphatic group(s), etc., or may be converted into the corresponding functional groups such as ethers, amines, or the like.

The antisense polynucleotide of the present invention is RNA, DNA or a modified nucleic acid (RNA, DNA). Specific examples of the modified nucleic acid are sulfur and thiophosphate derivatives of nucleic acids, those resistant to degradation of

polynucleoside amides or oligonucleoside amides, etc. The antisense nucleic acid of the present invention can be modified preferably based on the following design. The antisense nucleic acide of the present invention can be modified preferably based on the following design, that is, by increasing the intracellular stability of the antisense polynucleotide, enhancing the cell permeability of the antisense polynucleotide, increasing the affinity of the polynucleotide to the targeted sense strand to a higher level, or minimizing the toxicity, if any, of the antisense nucleic acide.

Many of such modifications are known and disclosed in, for example, Pharm. Tech. Japan, Vol. 8, p. 247 or 395, 1992, Antisense Research and Applications, CRC Press, 1993, etc.

The antisense nucleic acide of the present invention may contain altered or modified sugars, bases or linkages. The antisense nucleic acide may also be provided in a specialized form such as liposomes, microspheres, or may be applied to gene therapy, or may be provided in combination with attached moieties. Such attached moieties include polycations such as polylysine that act as charge neutralizers of the phosphate backbone, or hydrophobic moieties such as lipids (e.g., phospholipids, cholesterols, etc.) that enhance the interaction with cell membranes or increase uptake of the nucleic acid. Preferred examples of the lipids to be attached are cholesterols or derivatives thereof (e.g., cholesteryl chloroformate, cholic acid, etc.). These moieties may be attached to the nucleic acid at the 3' or 5' ends thereof and may also be attached thereto through a base, sugar, or intramolecular nucleoside linkage. Other moieties may be capping groups specifically placed at the 3' or 5' ends of the nucleic acid to prevent degradation by nucleases such as exonuclease, RNase, etc. Such capping groups include, but are not limited to, hydroxyl protecting groups known in the art, including glycols such as polyethylene glycol, tetraethylene glycol and the like.

The inhibitory activity of the antisense nucleic acide can be examined using the transformant of the present invention, the gene expression system of the present invention in vivo and in vitro, or the translation system for the protein of the present invention in vivo and in vitro. The nucleic acid can be applied to cells by various methods known per se.

Hereinafter, the protein of the present invention, its partial peptides, or salts thereof (hereinafter sometimes merely referred to as the protein of the present invention), the polynucleotide (e.g., DNA) (hereinafter sometimes merely referred to as the DNA of the present invention) encoding the protein of the present invention or its partial peptides, the antibodies against the protein of the present invention, its partial peptides, or salts

thereof (hereinafter sometimes referred to as the antibodies of the present invention) and the antisense polynucleotides to the polynucleotide (e.g., DNA) of the present invention (hereinafter sometimes merely referred to as the antisense polynucleotides of the present invention) are specifically described for their applications.

The protein comprising an amino acid sequence identical or substantially identical with the amino acid sequence represented by SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 30, SEQ ID NO: 32, SEQ ID NO: 34, SEQ ID NO: 36, SEQ ID NO: 38, SEQ ID NO: 40, SEQ ID NO: 42, SEQ ID NO: 44, SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 58, SEQ ID NO: 60 or SEQ ID NO: 62, or its partial peptide or a salt thereof is sometimes referred to "protein A of the present invention".

The protein comprising an amino acid sequence identical or substantially identical with the amino acid sequence represented by SEQ ID NO: 64 or SEQ ID NO: 66, or its partial peptide or a salt thereof is sometimes referred to "protein B of the present invention".

(1) Screening of pharmaceutical candidate compounds for disease

The protein A of the present invention is increasingly expressed in the lung as chronic obstructive pulmonary disease proceeds, and thus the compound or its salt that inhibits the activity of the protein A of the present invention can be used as a prophylactic/therapeutic agent for respiratory diseases [for example, chronic obstructive pulmonary disease (chronic bronchitis, pulmonary emphysema), diffuse panbronchiolitis, bronchial asthma, cystic fibrosis, hypersensitive pneumonia, pulmonary fibrosis etc.], preferably as a prophylactic/therapeutic agent for chronic obstructive pulmonary disease etc.

The protein A of the present invention is useful as a reagent for screening the compound or its salt that regulates (preferably inhibits) the activity of the protein A of the present invention.

The compound or its salt that regulates (preferably inhibits) the activity of the protein A of the present invention is screened by measuring and comparing the activity of the protein A of the present invention in the case (i) where a cell having an ability to produce the protein A of the present invention is cultured and in the case (ii) a cell having an ability to produce the protein A of the present invention is cultured in the

presence of a test compound.

As the cell having an ability to produce the protein A of the present invention, for example, a host (transformant) transformed with a vector containing the DNA encoding the protein of the present invention is used. Preferably, animal cells such as COS7 cells, CHO cells, HEK293 cells, etc. are used as the host. In the screening, it is preferable to use the transformant in which the protein A of the present invention has been expressed on the cell membrane or in the cells, e.g., by culturing through the procedures described above. The procedures for culturing the cells capable of expressing the protein A of the present invention are similar to the culturing procedures for the transformant of the present invention described above.

Examples of the test compound include peptides, proteins, antibodies, non-peptide compounds, synthetic compounds, fermentation products, cell extracts, plant extracts, animal tissue extracts, blood plasma etc.

For example, a test compound that decreases the activity of the protein A of the present invention in (ii) above by about at least 20%, preferably at least 30%, more preferably about at least 50% as compared with the activity in (i) above can be selected as a compound that inhibits the activity of the protein A of the present invention, while a test compound that increases the activity of the protein A of the present invention in (ii) above by about at least 20%, preferably at least 30%, more preferably about at least 50% as compared with the activity in (i) above can be selected as a compound that promotes the activity of the protein A of the present invention.

Hereinafter, the case where the protein A of the present invention is a protein comprising an amino acid sequence identical or substantially identical with the amino acid sequence represented by SEQ ID NO: 2, or its partial peptide or a salt thereof (abbreviated as protein A1 of the present invention) is described.

The protein A1 of the present invention produces 25-hydroxycholesterol from cholesterol in alveolar macrophage, and the 25-hydroxycholesterol promotes production of inflammatory cytokines (for example, CXCL2 and IL-1β) thereby accelerating neutrophil infiltration in the airway and advancing the morbid state of chronic obstructive pulmonary disease. Accordingly, the compound or its salt that inhibits the activity of the protein A1 of the present invention can be used for example as a prophylactic/therapeutic agent for respiratory diseases [for example, chronic obstructive pulmonary disease (chronic bronchitis, pulmonary emphysema), diffuse panbronchiolitis, bronchial asthma, cystic fibrosis, hypersensitive pneumonia, pulmonary fibrosis etc.].

Specifically, the screening method using the protein A1 of the present invention

includes a method of screening the compound or its salt that regulates (preferably inhibits) the activity of the protein A1 of the present invention, which comprises comparing (ia) the cholesterol hydroxylation activity of (the cell having an ability to produce) the protein A1 of the present invention, with (iia) the cholesterol hydroxylation activity of (the cell having an ability to produce) the protein A1 of the present invention in the presence of a test compound.

The cholesterol hydroxylation activity can be assayed by methods known per se, for example, a method described in J. Biol. Chem. 273:34316-34327 (1998) or with its modifications.

Specifically, the compound or its salt that regulates (preferably inhibits) the activity of the protein A1 of the present invention is screened by measuring the cholesterol hydroxylation activity in the case (ib) where the protein A1 of the present invention is reacted with a labeled cholesterol and in the case (iib) where the protein A1 of the present invention is reacted with a labeled cholesterol, in the presence of a test compound. This reaction is carried out in a suitable buffer. The cholesterol hydroxylation activity is determined by separating the product from the substrate by thin layer chromatography and then measuring the amount of the product (for example, radioactivity etc.). Measurement of the radioactivity is carried out according to a known method using a scintillation counter etc.

The protein A1 produced by culturing cells having an ability to produce the protein A1 of the present invention, cells having an ability to produce the protein A1 of the present invention, etc., are used as the protein A1 of the present invention. For example, the protein A1 expressed by cells obtained by inserting the base sequence represented by SEQ ID NO: 1 into a (commercial) expression vector for animal cell and then introducing it into animal cells (for example, COS cells), or cells obtained by inserting the base sequence represented by SEQ ID NO: 1 into an expression vector for animal cell and then introducing it into animal cells (for example, COS cells), etc., can be used.

The labeling agent used includes, radioisotopes (for example, [^{125}I], [^{131}I], [^{3}H], [^{14}C], [^{32}P], [^{33}P], [^{35}S], etc.), fluorescent substances [for example, cyanine fluorescent dyes (e.g., Cy2, Cy3, Cy5, Cy5.5, Cy7 (manufactured by Amersham Biosciences Corp.), etc.), fluorescamine, fluorescein isothiocyanate, NBD (7-nitrobenz-2-oxa-1,3-diazol), BODIPY (boron-dipyrromethene) etc.], enzymes (for example, β-galactosidase, β-glucosidase, alkaline phosphatase, peroxidase, malate dehydrogenase etc), luminescent substances (for example, luminol, a luminol derivative, luciferin, lucigenin etc.), biotin,

and lanthanide elements.

The screening method wherein a protein comprising an amino acid sequence identical or substantially identical with the amino acid sequence represented by SEQ ID NO: 30, or a partial peptide or its salt (abbreviated as protein A2 of the present invention) is used, for example, as the protein A of the present invention is described below,

Specific examples includes a method of screening the compound or its salt that regulates (preferably inhibits) the activity of the protein A2 of the present invention, which comprises comparing (ic) the proteolysis activity of the protein A2 of the present invention, with (iic) the proteolysis activity of the protein A2 of the present invention in the presence of a test compound.

The proteolysis hydrolysis activity can be assayed by methods known per se, for example, a method described in J. Biol. Chem. 272:4281-4286 (1997) or with its modifications.

Specifically, the compound or its salt that regulates (preferably inhibits) the activity of the protein A2 of the present invention is screened by measuring the proteolysis activity in the case (id) where the protein A2 of the present invention is reacted with a labeled substrate peptide and in the case (iid) where the protein A2 of the presence is reacted with a substrate peptide in the presence of a test compound. This reaction is carried out in a suitable buffer. By measuring the amount of the substrate peptide decomposed (for example, fluorescence intensity), the proteolysis activity is measured. As the labeled substrate peptide, use is made of, for example, a substrate peptide (for example, Nma-Pro-Lys-Pro-Leu-Ala-Nva-Trp-Lys (Dnp)-NH_2, Nma: N-methyl anthranilic acid etc.) labeled with a fluorescent substance (for example, fluorescamine, fluorescein isocyanate etc.). The fluorescence intensity is measured according to methods known in the art, for example a method using a fluorescence measuring apparatus.

As the protein A2 of the present invention, use is made of the one produced by culturing cells having an ability to produce the protein A2 of the present invention. For example, the base represented by SEQ ID NO: 29 is introduced into a commercial expression vector for prokaryotic cell, then introduced into a prokaryotic cell (for example, Escherichia coli), then expressed and re-folded to give the protein having the activity.

The protein B of the present invention is decreasingly expressed in the lung as chronic obstructive pulmonary disease proceeds, and thus the compound or its salt that promotes the activity of the protein B of the present invention can be used for example as

a prophylactic/therapeutic agent for respiratory diseases [for example, chronic obstructive pulmonary disease (chronic bronchitis, pulmonary emphysema), diffuse panbronchiolitis, bronchial asthma, cystic fibrosis, hypersensitive pneumonia, pulmonary fibrosis etc.], preferably as a prophylactic/therapeutic agent for chronic obstructive pulmonary disease etc.

The protein B of the present invention is useful as a reagent for screening the compound or its salt that regulates (preferably promotes) the activity of the protein B of the present invention.

The compound or its salt that regulates (preferably promotes) the activity of the protein B of the present invention is screened by measuring and comparing the activity of the protein B of the present invention in the case (i') where a cell having an ability to produce the protein B of the present invention is cultured and in the case (ii') where a cell having an ability to produce the protein B of the present invention is cultured in the presence of a test compound.

As the cell having an ability to produce the protein B of the present invention, for example, a host (transformant) transformed with a vector containing the DNA encoding the protein of the present invention is used. Preferably, animal cells such as COS7 cells, CHO cells, HEK293 cells, etc. are used as the host. In the screening, it is preferable to use the transformant in which the protein B of the present invention has been expressed on the cell membrane or in the cells, e.g., by culturing it through the procedures described above. The procedures for culturing the cells capable of expressing the protein B of the present invention are similar to the culturing procedures for the transformant of the present invention described above.

Examples of the test compound include peptides, proteins, antibodies, non-peptide compounds, synthetic compounds, fermentation products, cell extracts, plant extracts, animal tissue extracts, blood plasma etc.

For example, a test compound that increases the activity of the protein B of the present invention in (ii') above by about at least 20%, preferably at least 30%, more preferably about at least 50% as compared with the activity in (i') above can be selected as a compound that promotes the activity of the protein B of the present invention, while a test compound that decreases the activity of the protein B of the present invention in (ii') above by about at least 20%, preferably at least 30%, more preferably about at least 50% as compared with the activity in (i') above can be selected as a compound that inhibits the activity of the protein B of the present invention.

The gene encoding the protein A of the present invention is increasingly

expressed in the lung as chronic obstructive pulmonary disease proceeds, and thus the compound or its salt that inhibits the expression of the gene encoding the protein A of the present invention can be used as a prophylactic/therapeutic agent for respiratory diseases [for example, chronic obstructive pulmonary disease (chronic bronchitis, pulmonary emphysema), diffuse panbronchiolitis, bronchial asthma, cystic fibrosis, hypersensitive pneumonia, pulmonary fibrosis etc.], preferably as a prophylactic/therapeutic agent for chronic obstructive pulmonary disease etc.

The protein A1 of the present invention produces 25-hydroxycholesterol from cholesterol in alveolar macrophage, and the 25-hydroxycholesterol promotes production of inflammatory cytokines (for example, CXCL2 and IL-1β) thereby accelerating neutrophil infiltration in the airway and advancing the morbid state of chronic obstructive pulmonary disease. Accordingly, the compound or its salt that inhibits the expression of the gene encoding the protein A1 of the present invention can be used for example as a prophylactic/therapeutic agent for respiratory diseases [for example, chronic obstructive pulmonary disease (chronic bronchitis, pulmonary emphysema), diffuse panbronchiolitis, bronchial asthma, cystic fibrosis, hypersensitive pneumonia, pulmonary fibrosis etc.].

The gene encoding the protein B of the present invention is decreasingly expressed in the lung as chronic obstructive pulmonary disease proceeds, and thus the compound or its salt that regulates (preferably promotes) the expression of the gene encoding the protein B of the present invention can be used for example as a prophylactic/therapeutic agent for respiratory diseases [for example, chronic obstructive pulmonary disease (chronic bronchitis, pulmonary emphysema), diffuse panbronchiolitis, bronchial asthma, cystic fibrosis, hypersensitive pneumonia, pulmonary fibrosis etc.], preferably as a prophylactic/therapeutic agent for chronic obstructive pulmonary disease etc.

Therefore, the polynucleotide (for example, DNA) of the present invention is useful as a reagent for:

(a) screening the compound or its salt that regulates (preferably inhibits) the expression of the gene encoding the protein A of the present invention, or

(b) screening the compound or its salt that regulates (preferably promotes) the expression of the gene encoding the protein B of the present invention.

The screening method includes a method which comprises measuring and comparing the expression level of the gene (for example, the amount of the protein of the present invention or the amount of mRNA encoding the protein) in the case (iii) where cells having an ability to produce the protein of the present invention are cultured, with

that in the case (iv) where cells having an ability to produce the protein of the present invention are cultured in the presence of a test compound.

The test compound and the cells having an ability to produce the protein of the present invention include those described above.

In measuring the amount of the protein, the protein present in a cellular extract or the like can be measured according to known methods, for example by Western analysis, ELISA or the like, or a modification thereof, with antibodies recognizing the protein of the present invention.

The amount of mRNA can be measured according to known methods, for example by Northern hybridization using, as a probe, SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 37, SEQ ID NO: 39, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 57, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63 or SEQ ID NO: 65 or a nucleic acid comprising a part thereof, or by Northern hybridization using, as a primer, SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 37, SEQ ID NO: 39, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 57, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63 or SEQ ID NO: 65 or a nucleic acid comprising a part thereof, or by the PCR method using, as a primer, SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 37, SEQ ID NO: 39, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 57, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63 or SEQ ID NO: 65 or a nucleic acid comprising a part thereof, or by a modification thereof.

For example, when a test compound increases the expression level of the gene in the case (iv) described above by at least about 20%, preferably at least 30% and more preferably at least about 50%, as compared to the case (iii) above, the test compound can

be selected to be a compound promoting the expression of the gene encoding the protein of the present invention; when a test compound inhibits the expression level of the gene in the case (iv) described above by at least about 20%, preferably at least 30% and more preferably at least about 50%, as compared to the case (iii) above, the test compound can be selected to be a compound suppressing the expression of the gene encoding the protein of the present invention.

The screening kit of the present invention comprises the protein used in the present invention, the cell having an ability to produce the protein used in the present invention, the polynucleotide encoding the protein, or the like.

The compounds or salts thereof, which are obtainable using the screening method or screening kit of the present invention, are compounds (or salts thereof) selected from the above-mentioned test compounds, for example, peptides, proteins, antibodies, non-peptide compounds, synthetic compounds, fermentation products, cell extracts, plant extracts, animal tissue extracts, blood plasma, and the like, and these compounds or salts thereof are compounds or their salts regulating the activity (for example, scavenger receptor activity) of the protein of the present invention.

As the salts of the compounds, the same salts as those of the protein of the present invention can be used.

The compound or its salt that regulates (preferably inhibits) the activity of the protein A of the present invention, the compound or its salt that regulates (preferably inhibits) the expression of the gene encoding the protein A of the present invention, the compound or its salt that regulates (preferably promotes) the activity of the protein B of the present invention, and the compound or its salt that regulates (preferably promotes) the expression of the gene encoding the protein B of the present invention are low toxic and can be used for example as a prophylactic/therapeutic agent for respiratory diseases [for example, chronic obstructive pulmonary disease (chronic bronchitis, pulmonary emphysema), diffuse panbronchiolitis, bronchial asthma, cystic fibrosis, hypersensitive pneumonia, pulmonary fibrosis etc.], preferably as a prophylactic/therapeutic agent for chronic obstructive pulmonary disease etc.

Where the compound or its salts obtained by using the screening method or screening kit of the present invention are used as the prophylactic/therapeutic agents described above, these compounds can be converted into pharmaceutical preparations in a conventional manner.

For example, the composition for oral administration includes solid or liquid preparations, specifically tablets (including dragees and film-coated tablets), pills,

granules, powdery preparations, capsules (including soft capsules), syrup, emulsions, suspensions, etc. Such a composition is manufactured by publicly known methods and contains a carrier, a diluent or excipient conventionally used in the field of pharmaceutical preparations. Examples of the carrier or excipient for tablets are lactose, starch, sucrose, magnesium stearate, etc.

Examples of the composition for parenteral administration are injectable preparations, suppositories, etc. The injectable preparations may include dosage forms such as intravenous, subcutaneous, intracutaneous and intramuscular injections, drip infusions, intraarticular injection, etc. These injectable preparations may be prepared by methods publicly known. For example, the injectable preparations may be prepared by dissolving, suspending or emulsifying the antibody or its salt described above in a sterile aqueous medium or an oily medium conventionally used for injections. As the aqueous medium for injections, there are for example physiological saline, an isotonic solution containing glucose and other auxiliary agents, etc., which may be used in combination with an appropriate solubilizing agent such as an alcohol (e.g., ethanol), a polyalcohol (e.g., propylene glycol, polyethylene glycol), a nonionic surfactant [e.g., polysorbate 80, HCO-50 (polyoxyethylene (50 mols) adduct of hydrogenated castor oil)], etc. As the oily medium, there are employed e.g., sesame oil, soybean oil, etc., which may be used in combination with a solubilizing agent such as benzyl benzoate, benzyl alcohol, etc. The injection thus prepared is usually filled in an appropriate ampoule. The suppository used for rectal administration may be prepared by blending the aforesaid antibody or its salt with conventional bases for suppositories.

Advantageously, the pharmaceutical compositions for oral or parenteral use described above are prepared into pharmaceutical preparations with a unit dose suited to fit a dose of the active ingredients. Such unit dose preparations include, for example, tablets, pills, capsules, injections (ampoules), suppositories, etc. The amount of the aforesaid compound contained is generally 5 to 500 mg per dosage unit form; it is preferred that the aforesaid antibody is contained in about 5 to about 100 mg especially in the form of injection, and in 10 to 250 mg for the other forms.

Each composition described above may further contain other active components unless formulation causes any adverse interaction with the compound described above.

Since the pharmaceutical preparations thus obtained are safe and low toxic, they can be administered to human or warm-blooded animal (e.g., mouse, rat, rabbit, sheep, swine, bovine, equine, fowl, feline, canine, simian, chimpanzee, etc.) orally or parenterally.

The dose of the compound or its salts may vary depending upon its action, target disease, subject to be administered, route of administration, etc. For example, when the compound or its salt that inhibits the activity of the protein A of the present invention is orally administered for the purpose of treating pulmonary emphysema, the compound or its salt is generally administered to an adult (as 60 kg body weight) in a daily dose of about 0.1 to about 100 mg, preferably about 1.0 to about 50 mg and more preferably about 1.0 to about 20 mg. In parenteral administration, a single dose of the said compound or its salt may vary depending upon subject to be administered, target disease, etc. When the compound or its salt that inhibits the activity of the protein A of the present invention is administered to an adult (as 60 kg body weight) in the form of an injectable preparation for the purpose of treating pulmonary emphysema, for example, it is advantageous to administer the compound or its salt by way of intravenous injection in a daily dose of about 0.01 to about 30 mg, preferably about 0.1 to about 20 mg, and more preferably about 0.1 to about 10 mg. For other animal species, the corresponding dose as converted per 60 kg weight can be administered.

(2) Quantification for the protein of the present invention, its partial peptide or salts thereof

The antibody against the protein of the present invention (hereinafter sometimes merely referred to as the antibody of the present invention) is capable of specifically recognizing the protein of the present invention, and thus can be used for quantification of the protein of the present invention in a test sample fluid, in particular, for quantification by sandwich immunoassay; etc.

That is, the present invention provides:

(i) a method of quantifying the protein of the present invention in a test sample fluid, which comprises competitively reacting the antibody of the present invention, a test sample fluid and a labeled form of the protein of the present invention, and measuring the ratio of the labeled form of the protein of the present invention bound to said antibody; and,

(ii) a method of quantifying the protein of the present invention in a test sample fluid, which comprises reacting a test sample fluid simultaneously or continuously with the antibody of the present invention immobilized on a carrier and another labeled antibody of the present invention, and then measuring the activity of the labeling agent on the insoluble carrier.

In the quantification method (ii) described above, it is preferred that one

antibody is capable of recognizing the N-terminal region of the protein of the present invention, while another antibody is capable of reacting with the C-terminal region of the protein of the present invention.

The monoclonal antibody against the protein of the present invention (hereinafter sometimes referred to as the monoclonal antibody of the present invention) can be used to quantify the protein of the present invention. In addition, the protein can be detected by means of a tissue staining as well. For these purposes, the antibody molecule per se may be used or $F(ab')_2$, Fab' or Fab fractions of the antibody molecule may also be used.

The method of quantifying the protein of the present invention using the antibody of the present invention is not particularly limited. Any quantification method can be used, so long as the amount of antibody, antigen or antibody-antigen complex corresponding to the amount of antigen (e.g., the amount of the protein) in a test sample fluid can be detected by chemical or physical means and the amount of the antigen can be calculated from a standard curve prepared from standard solutions containing known amounts of the antigen. For such an assay method, for example, nephrometry, the competitive method, the immunometric method, the sandwich method, etc. are suitably used and in terms of sensitivity and specificity, it is particularly preferred to use the sandwich method described hereinafter.

Examples of the labeling agent used in the assay method using the labeling substance are radioisotopes, enzymes, fluorescent substances, luminescent substances, lanthanide, and the like. As the radioisotopes, there are used, e.g., [^{125}I], [^{131}I], [^{3}H], [^{14}C], etc. The enzymes described above are preferably enzymes, which are stable and have a high specific activity, and include, e.g., β-galactosidase, β-glucosidase, an alkaline phosphatase, a peroxidase, malate dehydrogenase, etc. As the fluorescent substances, there are used, e.g., fluorescamine, fluorescein isothiocyanate, etc. As the luminescent substances described above there are used, e.g., luminol, a luminol derivative, luciferin, lucigenin, etc. Furthermore, the biotin-avidin system may be used as well for binding of an antibody or antigen to a labeling agent.

For immobilization of the antigen or antibody, physical adsorption may be used. Chemical binding techniques conventionally used for insolubilization or immobilization of proteins, enzymes, etc. may also be used. For carriers, there are used, e.g., insoluble polysaccharides such as agarose, dextran, cellulose, etc.; synthetic resin such as polystyrene, polyacrylamide, silicon, etc., and glass or the like.

In the sandwich method, the immobilized monoclonal antibody of the present

invention is reacted with a test fluid (primary reaction), then with a labeled form of another monoclonal antibody of the present invention (secondary reaction), and the activity of the label on the immobilizing carrier is measured, whereby the amount of the protein of the present invention in the test fluid can be quantified. The order of the primary and secondary reactions may be reversed, and the reactions may be performed simultaneously or with an interval. The methods of labeling and immobilization can be performed by the methods described above. In the immunoassay by the sandwich method, the antibody used for immobilized or labeled antibodies is not necessarily one species, but a mixture of two or more species of antibody may be used to increase the measurement sensitivity.

In the methods of assaying the protein of the present invention by the sandwich method of the present invention, antibodies that bind to different sites of the protein of the present invention are preferably used as the monoclonal antibodies of the present invention used for the primary and secondary reactions. That is, in the antibodies used for the primary and secondary reactions are, for example, when the antibody used in the secondary reaction recognizes the C-terminal region of the protein of the present invention, it is preferable to use the antibody recognizing the region other than the C-terminal region for the primary reaction, e.g., the antibody recognizing the N-terminal region.

The monoclonal antibodies of the present invention can be used for the assay systems other than the sandwich method, for example, the competitive method, the immunometric method, nephrometry, etc.

In the competitive method, antigen in a test fluid and the labeled antigen are competitively reacted with antibody, and the unreacted labeled antigen (F) and the labeled antigen bound to the antibody (B) are separated (B/F separation). The amount of the label in B or F is measured, and the amount of the antigen in the test fluid is quantified. This reaction method includes a liquid phase method using a soluble antibody as an antibody, polyethylene glycol for B/F separation and a secondary antibody against the soluble antibody, and an immobilized method either using an immobilized antibody as the primary antibody, or using a soluble antibody as the primary antibody and immobilized antibody as the secondary antibody.

In the immunometric method, antigen in a test fluid and immobilized antigen are competitively reacted with a definite amount of labeled antibody, the immobilized phase is separated from the liquid phase, or antigen in a test fluid and an excess amount of labeled antibody are reacted, immobilized antigen is then added to bind the unreacted

labeled antibody against the immobilized phase, and the immobilized phase is separated from the liquid phase. Then, the amount of the label in either phase is measured to quantify the antigen in the test fluid.

In the nephrometry, insoluble precipitate produced after the antigen-antibody reaction in gel or solution is quantified. When the amount of antigen in the test fluid is small and only a small amount of precipitate is obtained, laser nephrometry using scattering of laser is advantageously employed.

For applying each of these immunological methods to the quantification method of the present invention, any particular conditions or procedures are not required. Quantification system for the protein of the present invention or its salts is constructed by adding the usual technical consideration in the art to the conventional conditions and procedures. For the details of these general technical means, reference can be made to the following reviews and texts.

For example, Hiroshi Irie, ed. "Radioimmunoassay" (Kodansha, published in 1974), Hiroshi Irie, ed. "Sequel to the Radioimmunoassay" (Kodansha, published in 1979), Eiji Ishikawa, et al. ed. "Enzyme immunoassay" (Igakushoin, published in 1978), Eiji Ishikawa, et al. ed. "Immunoenzyme assay" (2nd ed.) (Igakushoin, published in 1982), Eiji Ishikawa, et al. ed. "Immunoenzyme assay" (3rd ed.) (Igakushoin, published in 1987), Methods in ENZYMOLOGY, Vol. 70 (Immunochemical Techniques (Part A)), ibid., Vol. 73 (Immunochemical Techniques (Part B)), ibid., Vol. 74 (Immunochemical Techniques (Part C)), ibid., Vol. 84 (Immunochemical Techniques (Part D: Selected Immunoassays)), ibid., Vol. 92 (Immunochemical Techniques (Part E: Monoclonal Antibodies and General Immunoassay Methods)), ibid., Vol. 121 (Immunochemical Techniques (Part I: Hybridoma Technology and Monoclonal Antibodies)) (all published by Academic Press Publishing).

As described above, the protein of the present invention can be quantified with high sensitivity, using the antibody of the present invention.

Furthermore, when an increased or decreased level of the protein of the present invention is detected by quantifying the concentration of the protein of the present invention using the antibody of the present invention, it can be diagnosed that one suffers from, for example, respiratory diseases [for example, chronic obstructive pulmonary disease (chronic bronchitis, pulmonary emphysema), diffuse panbronchiolitis, bronchial asthma, cystic fibrosis, hypersensitive pneumonia, pulmonary fibrosis etc.]; or it is highly likely to suffer from these disease in the future.

In addition, the antibody of the present invention can be used to detect the

protein of the present invention, which is present in a test sample such as a body fluid, a tissue, etc. The antibody can also be used to prepare an antibody column for purification of the protein of the present invention, detect the protein of the present invention in each fraction upon purification, analyze the behavior of the protein of the present invention in the cells under investigation; etc.

(3) Gene diagnostic agent

By using the DNA of the present invention, e.g., as a probe, the DNA can detect an abnormality (gene abnormality) of the DNA or mRNA encoding the protein of the present invention or its partial peptide in human or warm-blooded animal (e.g., rat, mouse, guinea pig, rabbit, fowl, ovine, swine, bovine, equine, feline, canine, simian, chimpanzee, etc.). Therefore, the DNA of the present invention is useful as a gene diagnostic agent for detecting damages to the DNA or mRNA, its mutation, or decreased expression, increased expression, overexpression, etc. of the DNA or mRNA, and so on.

The gene diagnosis described above using the DNA of the present invention can be performed by, for example, the publicly known Northern hybridization assay or the PCR-SSCP assay (Genomics, 5, 874-879 (1989); Proceedings of the National Academy of Sciences of the United States of America, 86, 2766-2770 (1989)), etc.

When overexpression or decreased expression is detected by, e.g., Northern hybridization or DNA mutation is detected by the PCR-SSCP assay, it can be diagnosed that it is highly likely to suffer from, for example, respiratory diseases [for example, chronic obstructive pulmonary disease (chronic bronchitis, pulmonary emphysema), diffuse panbronchiolitis, bronchial asthma, cystic fibrosis, hypersensitive pneumonia, pulmonary fibrosis etc.].

(4) Pharmaceutical preparation comprising the antisense polynucleotide

The antisense polynucleotide of the present invention that binds complementarily to the polynucleotide (for example, DNA) encoding the protein A of the present invention to inhibit expression of the DNA is low-toxic and can suppress the functions and activity of the protein A or DNA encoding the protein in the body, and can thus be used for example as a prophylactic/therapeutic agent for respiratory diseases [for example, chronic obstructive pulmonary disease (chronic bronchitis, pulmonary emphysema), diffuse panbronchiolitis, bronchial asthma, cystic fibrosis, hypersensitive pneumonia, pulmonary fibrosis etc.], preferably a prophylactic/therapeutic agent for chronic obstructive pulmonary disease etc.

Where the antisense polynucleotide described above is used as the aforesaid prophylactic/therapeutic agent, it can be prepared into pharmaceutical preparations by publicly known methods, which are provided for administration.

For example, when the antisense polynucleotide described above is used, the antisense polynucleotide alone is administered directly, or the antisense polynucleotide is inserted into an appropriate vector such as retrovirus vector, adenovirus vector, adenovirus-associated virus vector, etc., followed by treating in a conventional manner. The antisense polynucleotide may then be administered orally or parenterally to human or mammal (e.g., rat, rabbit, ovine, swine, bovine, feline, canine, simian, etc.) in a conventional manner. The antisense polynucleotide may also be administered as it stands, or may be prepared in pharmaceutical preparations together with a physiologically acceptable carrier to assist its uptake, which are then administered by gene gun or through a catheter such as a catheter with a hydrogel. Alternatively, the antisense polynucleotide may be prepared into an aerosol, which is topically administered into the trachea as an inhaler.

Further for the purposes of improving pharmacokinetics, extending a half-life and improving intracellular uptake efficiency, the antisense polynucleotide described above is prepared into pharmaceutical preparations (injectable preparations) alone or together with a carrier such as liposome, etc. and the preparations may be administered intravenously, subcutaneously, in airway or at the pulmonary regions, etc.

A dose of the antisense polynucleotide may vary depending on target disease, subject to be administered, route for administration, etc. For example, where the antisense polynucleotide of the present invention is administered for the purpose of treating pulmonary emphysema, the antisense polynucleotide is generally administered to an adult (60 kg body weight) in a daily dose of about 0.1 to 100 mg.

Similar to the antisense polynucleotide, the double-stranded RNA (for example, siRNA (small (short) interfering RNA) or shRNA (small (short) hairpin RNA to the polynucleotide encoding the protein A of the present invention) comprising a part of RNA encoding the protein A of the present invention, and the ribozyme comprising a part of RNA encoding the protein A of the present invention, can inhibit the expression of the gene encoding the protein, and can suppress the functions of the protein A or DNA encoding the protein in the body, and can thus be used for example as a prophylactic/therapeutic agent for respiratory diseases [for example, chronic obstructive pulmonary disease (chronic bronchitis, pulmonary emphysema), diffuse panbronchiolitis, bronchial asthma, cystic fibrosis, hypersensitive pneumonia, pulmonary fibrosis etc.],

preferably a prophylactic/therapeutic agent for chronic obstructive pulmonary disease etc.

The double-stranded RNA can be designed based on a sequence of the polynucleotide of the present invention and manufactured by modifications of publicly known methods (e.g., Nature, 411, 494, 2001).

The ribozyme can be designed based on a sequence of the polynucleotide of the present invention and manufactured by modifications of publicly known methods (e.g., TRENDS in Molecular Medicine, 7, 221, 2001). For example, the ribozyme can be manufactured by ligating a publicly known ribozyme to a part of the RNA encoding the protein of the present invention. A part of the RNA encoding the protein of the present invention includes a portion proximal to a cleavage site on the RNA of the present invention, which may be cleaved by a publicly known ribozyme (RNA fragment). Where the double-stranded RNA or the ribozyme described above can be used for example as a prophylactic/therapeutic agent,, it can be prepared into pharmaceutical preparations similar to the antisense polynucleotide, which are provided for administration.

(5) Pharmaceutical preparation comprising the antibody of the present invention

The antibody of the present invention can be used for example as a prophylactic/therapeutic agent for respiratory diseases [for example, chronic obstructive pulmonary disease (chronic bronchitis, pulmonary emphysema), diffuse panbronchiolitis, bronchial asthma, cystic fibrosis, hypersensitive pneumonia, pulmonary fibrosis etc.], preferably a prophylactic/therapeutic agent for chronic obstructive pulmonary disease etc.

The antibody of the present invention can be administered directly or as a pharmaceutical composition of appropriate dosage form. The pharmaceutical composition used for the administration comprises the antibody described above or its salt, pharmaceutically acceptable carriers, dilutes or excipients. Such a composition is provided as a dosage form appropriate for oral or parenteral administration.

For example, the composition for oral administration includes solid or liquid preparations, specifically tablets (including dragees and film-coated tablets), pills, granules, powdery preparations, capsules (including soft capsules), syrup, emulsions, suspensions, etc. Such a composition is manufactured by publicly known methods and contains a carrier, a diluent or excipient conventionally used in the field of pharmaceutical preparations. Examples of the carrier or excipient for tablets are

lactose, starch, sucrose, magnesium stearate, etc.

Examples of the composition for parenteral administration are injectable preparations, suppositories, etc. The injectable preparations may include dosage forms such as intravenous, subcutaneous, intracutaneous, intramuscular injections, drip infusions, etc.

These injectable preparations may be prepared by methods publicly known. For example, the injectable preparations may be prepared by dissolving, suspending or emulsifying the antibody or its salt described above in a sterile aqueous medium or an oily medium conventionally used for injections. As the aqueous medium for injections, there are for example physiological saline, an isotonic solution containing glucose and other auxiliary agents, etc., which may be used in combination with an appropriate solubilizing agent such as an alcohol (e.g., ethanol), a polyalcohol (e.g., propylene glycol, polyethylene glycol), a nonionic surfactant [e.g., polysorbate 80, HCO-50 (polyoxyethylene (50 mols) adduct of hydrogenated castor oil)], etc. As the oily medium, there are employed e.g. sesame oil, soybean oil, etc., which may be used in combination with a solubilizing agent such as benzyl benzoate, benzyl alcohol, etc. The injection thus prepared is usually filled in an appropriate ampoule. The suppository used for rectal administration may be prepared by blending the aforesaid antibody or its salt with conventional bases for suppositories.

Advantageously, the pharmaceutical compositions for oral or parenteral use described above are prepared into pharmaceutical preparations with a unit dose suited to fit a dose of the active ingredients. Such unit dose preparations include, for example, tablets, pills, capsules, injections (ampoules), suppositories, etc. The amount of the aforesaid compound contained is generally 5 to 500 mg per dosage unit form; it is preferred that the aforesaid antibody is contained in about 5 to about 100 mg especially in the form of injection, and in 10 to 250 mg for the other forms.

Each composition described above may further contain other active components unless formulation causes any adverse interaction with the compound described above.

The prophylactic/therapeutic agent comprising the antibody of the present invention for the diseases described above is low toxic and can be administered to human or mammals (e.g., rats, rabbits, ovine, swine, bovine, feline, canine, simian, etc.) orally or parenterally (e.g., intravenously) in the form of liquid preparation as it is or as a pharmaceutical composition of appropriate dosage form. The dose may vary depending upon subject to be administered, target disease, conditions, route of administration, etc. For example, when the agent is used for the purpose of treating, e.g., breast cancer in an

adult, it is advantageous to administer the antibody of the present invention normally in a single dose of about 0.01 to about 20 mg/kg body weight, preferably about 0.1 to about 10 mg/kg body weight, and more preferably about 0.1 to about 5 mg/kg body weight, approximately 1 to 5 times per day, preferably approximately 1 to 3 times per day. In other parenteral administration and oral administration, the agent can be administered in a dose corresponding to the dose given above. When the condition is especially severe, the dose may be increased according to the condition.

The antibody of the present invention is also useful for example as a diagnostic agent for respiratory diseases [for example, chronic obstructive pulmonary disease (chronic bronchitis, pulmonary emphysema), diffuse panbronchiolitis, bronchial asthma, cystic fibrosis, hypersensitive pneumonia, pulmonary fibrosis etc.].

(6) Prophylactic/therapeutic agent for diseases in which the protein of the present invention is involved

The protein B of the present invention is decreasingly expressed in the lung as chronic obstructive pulmonary disease proceeds. When the protein B of the present invention or the polynucleotide encoding the same is abnormal or deficient, it is highly likely to suffer from respiratory diseases [for example, chronic obstructive pulmonary disease (chronic bronchitis, pulmonary emphysema), diffuse panbronchiolitis, bronchial asthma, cystic fibrosis, hypersensitive pneumonia, pulmonary fibrosis etc.]. Accordingly, the protein B of the present invention or the polynucleotide encoding the same can be used for example as a prophylactic/therapeutic agent for respiratory diseases [for example, chronic obstructive pulmonary disease (chronic bronchitis, pulmonary emphysema), diffuse panbronchiolitis, bronchial asthma, cystic fibrosis, hypersensitive pneumonia, pulmonary fibrosis etc.].

For example, when there is a patient showing a decrease or deficiency in the protein B of the present invention or the polynucleotide encoding the same in the living body, (A) the polynucleotide is administered into the patient to express the protein B of the invention in the living body, (B) the polynucleotide is inserted into cells to express the protein B of the invention and the cells are transplanted to the patient, or (C) the protein B of the invention is administered into the patient, whereby the role of the protein B of the invention can be exhibited sufficiently or normally in the patient.

Where the polynucleotide (for example, DNA) described above is used as the prophylactic/therapeutic agents described above, the DNA itself is administered directly to human or other warm-blooded animal; alternatively, the DNA is inserted into an

appropriate vector such as retrovirus vector, adenovirus vector, adenovirus-associated virus vector, etc. and then administered to human or other warm-blooded animal in a conventional manner. The DNA of the present invention may also be administered as an intact DNA, or prepared into medicines together with physiologically acceptable carriers such as adjuvants to assist its uptake, which are administered by gene gun or through a catheter such as a hydrogel catheter.

Where the protein B of the present invention is used as the aforesaid prophylactic/therapeutic agents, the protein is advantageously used on a purified level of at least 90%, preferably at least 95%, more preferably at least 98% and most preferably at least 99%.

The protein B of the present invention can be used orally, for example, in the form of tablets which may be sugar coated if necessary and desired, capsules, elixirs, microcapsules etc., or parenterally (preferably subcutaneously) in the form of injectable preparations such as a sterile solution and a suspension in water or with other pharmaceutically acceptable liquid. For example, these preparations can be manufactured by mixing the protein B of the present invention with a physiologically acceptable known carrier, a flavoring agent, an excipient, a vehicle, an antiseptic agent, a stabilizer, a binder, etc. in a unit dosage form required in a generally accepted manner that is applied to making medicines. The active ingredient in the preparation is controlled in such a dose that an appropriate dose is obtained within the specified range given.

A vector into which the polypeptide (for example, DNA) was inserted can be prepared into a pharmaceutical preparation in a conventional manner and used usually parenterally.

Since the pharmaceutical preparation thus obtained is safe and low toxic, it can be administered to human or mammal (e.g., rat, mouse, guinea pig, rabbit, bird, ovine, swine, bovine, equine, feline, canine, simian, etc.).

The dose of the protein B of the present invention may vary depending upon target disease, subject to be administered, route of administration, etc. For example, when the protein B of the present invention is parenterally administered for the purpose of treating pulmonary emphysema, it can be administered to the adult patient (as 60 kg body weight) generally in a dose of about 0.1 to 100 mg, preferably about 1.0 to 50 mg and more preferably about 1.0 to 20 mg. For other animal species, the corresponding dose as converted per 60 kg weight can be administered.

(7) With respect to "Prophylactic/therapeutic agent for respiratory diseases comprising the compound or its salt that has an action of regulating the cholesterol hydroxylation activity"

The "compound having the action of regulating the cholesterol hydroxylation activity may be any compounds having the action of regulating the cholesterol hydroxylation activity (for example, peptides, proteins, antibodies, non-peptide compounds, synthetic compounds, fermentation products, cell extracts, plant extracts, animal tissue extracts, blood plasma, etc.), and can be used for example as a prophylactic/therapeutic agent for respiratory diseases [for example, chronic obstructive pulmonary disease (chronic bronchitis, pulmonary emphysema), diffuse panbronchiolitis, bronchial asthma, cystic fibrosis, hypersensitive pneumonia, pulmonary fibrosis etc.], preferably as a prophylactic/therapeutic agent for chronic obstructive pulmonary disease etc.

The prophylactic/therapeutic agent can be produced in the same manner as described above.

(8) DNA transgenic animal

The present invention provides a non-human mammal bearing DNA encoding the protein of the present invention, which is exogenous (hereinafter abbreviated as the exogenous DNA of the present invention) or its variant DNA (sometimes simply referred to as the exogenous variant DNA of the present invention).

That is, the present invention provides:

(1) A non-human mammal bearing the exogenous DNA of the present invention or its variant DNA;

(2) The mammal according to (1), wherein the non-human mammal is a rodent;

(3) The mammal according to (2), wherein the rodent is mouse or rat; and,

(4) A recombinant vector containing the exogenous DNA of the present invention or its variant DNA and capable of expressing in a mammal; etc.

The non-human mammal bearing the exogenous DNA of the present invention or its variant DNA (hereinafter simply referred to as the DNA transgenic animal of the present invention) can be prepared by transfecting a desired DNA into an unfertilized egg, a fertilized egg, a spermatozoon, a germinal cell containing a primordial germinal cell thereof, or the like, preferably in the embryogenic stage in the development of a non-human mammal (more preferably in the single cell or fertilized cell stage and generally before the 8-cell phase), by standard means, such as the calcium phosphate

method, the electric pulse method, the lipofection method, the agglutination method, the microinjection method, the particle gun method, the DEAE-dextran method, etc. Also, it is possible to transfect the exogenous DNA of the present invention into a somatic cell, a living organ, a tissue cell, or the like by the DNA transfection methods, and to utilize the transformant for cell culture, tissue culture, etc. In addition, these cells may be fused with the above-described germinal cell by a publicly known cell fusion method to prepare the DNA transgenic animal of the present invention.

Examples of the non-human mammal that can be used include bovine, swine, ovine, goat, rabbits, canine, feline, guinea pigs, hamsters, mice, rats, etc. Above all, preferred are rodents, especially mice (e.g., C57Bl/6 strain, DBA2 strain, etc. for a pure line and for a cross line, B6C3F1 strain, BDF1 strain B6D2F1 strain, BALB/c strain, ICR strain, etc.), rats (Wistar, SD, etc.) or the like, since they are relatively short in ontogeny and life cycle from a standpoint of creating model animals for human disease.

"Mammals" in a recombinant vector that can be expressed in the mammals include the aforesaid non-human mammals, human, etc.

The exogenous DNA of the present invention refers to the DNA of the present invention that is once isolated/extracted from mammals, not the DNA of the present invention inherently possessed by the non-human mammals.

The mutant DNA of the present invention includes mutants resulting from variation (e.g., mutation, etc.) in the base sequence of the original DNA of the present invention, specifically DNAs resulting from base addition, deletion, substitution with other bases, etc. and further including abnormal DNA.

The abnormal DNA is intended to mean DNA that expresses the protein of the present invention which is abnormal and exemplified by the DNA, etc. that expresses a protein for suppressing the function of the protein of the present invention which is normal.

The exogenous DNA of the present invention may be any one of those derived from a mammal of the same species as, or a different species from, the mammal as the target animal. In transfecting the DNA of the present invention into the target animal, it is generally advantageous to use the DNA as a DNA construct in which the DNA is ligated downstream a promoter capable of expressing the DNA in the target animal. For example, in the case of transfecting the human DNA of the present invention, a DNA transgenic mammal that expresses the DNA of the present invention to a high level, can be prepared by microinjecting a DNA construct (e.g., vector, etc.) ligated with the human DNA of the present invention into a fertilized egg of the target non-human mammal

downstream various promoters which are capable of expressing the DNA derived from various mammals (e.g., rabbits, canine, feline, guinea pigs, hamsters, rats, mice, etc.) bearing the DNA of the present invention highly homologous to the human DNA.

As expression vectors for the protein of the present invention, there are Escherichia coli-derived plasmids, Bacillus subtilis-derived plasmids, yeast-derived plasmids, bacteriophages such as λ phage, retroviruses such as Moloney leukemia virus, etc., and animal viruses such as vaccinia virus, baculovirus, etc. Of these vectors, Escherichia coli-derived plasmids, Bacillus subtilis-derived plasmids, or yeast-derived plasmids, etc. are preferably used.

Examples of these promoters for regulating the DNA expression described above include (i) promoters for DNA derived from viruses (e.g., simian virus, cytomegalovirus, Moloney leukemia virus, JC virus, breast cancer virus, poliovirus, etc.), and (ii) promoters derived from various mammals (human, rabbits, canine, feline, guinea pigs, hamsters, rats, mice, etc.), for example, promoters of albumin, insulin II, uroplakin II, elastase, erythropoietin, endothelin, muscular creatine kinase, glial fibrillary acidic protein, glutathione S-transferase, platelet-derived growth factor β, keratins K1, K10 and K14, collagen types I and II, cyclic AMP-dependent protein kinase βI subunit, dystrophin, tartarate-resistant alkaline phosphatase, atrial natriuretic factor, endothelial receptor tyrosine kinase (generally abbreviated as Tie2), sodium-potassium adenosine triphosphorylase (Na,K-ATPase), neurofilament light chain, metallothioneins I and IIA, metalloproteinase I tissue inhibitor, MHC class I antigen (H-2L), H-ras, renin, dopamine β-hydroxylase, thyroid peroxidase (TPO), protein chain elongation factor 1α (EF-1α), β actin, α and β myosin heavy chains, myosin light chains 1 and 2, myelin base protein, thyroglobulins, Thy-1, immunoglobulins, H-chain variable region (VNP), serum amyloid component P, myoglobin, troponin C, smooth muscle α actin, preproencephalin A, vasopressin, etc. Among them, cytomegalovirus promoters, human protein elongation factor 1α (EF-1α) promoters, human and fowl β actin promoters, etc., which are capable of high expression in the whole body are preferred.

Preferably, the vectors described above have a sequence that terminates the transcription of the desired messenger RNA in the DNA transgenic animal (generally termed a terminator); for example, a sequence of each DNA derived from viruses and various mammals, and SV40 terminator of the simian virus and the like are preferably used.

In addition, for the purpose of enhancing the expression of the desired exogenous DNA to a higher level, the splicing signal and enhancer region of each DNA,

a portion of the intron of an eukaryotic DNA may also be ligated at the 5' upstream of the promoter region, or between the promoter region and the translational region, or at the 3' downstream of the translational region, depending upon purposes.

The translational region for the normal protein of the present invention can be obtained using as a starting material the entire genomic DNA or its portion of liver, kidney, thyroid cell or fibroblast origin from human or various mammals (e.g., rabbits, canine, feline, guinea pigs, hamsters, rats, mice, etc.) or of various commercially available genomic DNA libraries, or using cDNA prepared by a publicly known method from RNA of liver, kidney, thyroid cell or fibroblast origin as a starting material. Also, an exogenous abnormal DNA can produce the translational region through variation of the translational region of normal protein obtained from the cells or tissues described above by point mutagenesis.

The translational region can be prepared by a conventional DNA engineering technique, in which the DNA is ligated downstream the aforesaid promoter and if desired, upstream the translation termination site, as a DNA construct capable of being expressed in the transgenic animal.

The exogenous DNA of the present invention is transfected at the fertilized egg cell stage in a manner such that the DNA is certainly present in all the germinal cells and somatic cells of the target mammal. The fact that the exogenous DNA of the present invention is present in the germinal cells of the animal prepared by DNA transfection means that all offspring of the prepared animal will maintain the exogenous DNA of the present invention in all of the germinal cells and somatic cells thereof. The offspring of the animal that inherits the exogenous DNA of the present invention also have the exogenous DNA of the present invention in all of the germinal cells and somatic cells thereof.

The non-human mammal in which the normal exogenous DNA of the present invention has been transfected can be passaged as the DNA-bearing animal under ordinary rearing environment, by confirming that the exogenous DNA is stably retained by crossing.

By the transfection of the exogenous DNA of the present invention at the fertilized egg cell stage, the DNA is retained to be excess in all of the germinal and somatic cells. The fact that the exogenous DNA of the present invention is excessively present in the germinal cells of the prepared animal after transfection means that the DNA of the present invention is excessively present in all of the germinal cells and somatic cells thereof. The offspring of the animal that inherits the exogenous DNA of

the present invention have excessively the DNA of the present invention in all of the germinal cells and somatic cells thereof.

It is possible to obtain homozygotic animals having the transfected DNA in both homologous chromosomes and breed male and female of the animal so that all the progeny have this DNA in excess.

In a non-human mammal bearing the normal DNA of the present invention, the normal DNA of the present invention has expressed at a high level, and may eventually develop hyperfunction in the function of the protein of the present invention by accelerating the function of endogenous normal DNA. Therefore, the animal can be utilized as a pathologic model animal for such a disease. For example, using the normal DNA transgenic animal of the present invention, it is possible to elucidate the mechanism of hyperfunction in the function of the protein of the present invention and the pathological mechanism of the disease associated with the protein of the present invention and to investigate how to treat these diseases.

The mammal in which the normal exogenous DNA of the present invention has been transfected has a symptom of increase or decrease in the protein of the present invention in a free form and can thus be applied for example to a test for screening of a prophylactic/therapeutic agent for respiratory diseases [for example, chronic obstructive pulmonary disease (chronic bronchitis, pulmonary emphysema), diffuse panbronchiolitis, bronchial asthma, cystic fibrosis, hypersensitive pneumonia, pulmonary fibrosis etc.].

On the other hand, a non-human mammal having the exogenous abnormal DNA of the present invention can be passaged under normal breeding conditions as the DNA-bearing animal by confirming stable retention of the exogenous DNA via crossing. Furthermore, the exogenous DNA of interest can be utilized as a starting material by inserting the DNA into the plasmid described above. The DNA construct with a promoter can be prepared by conventional DNA engineering techniques. The transfection of the abnormal DNA of the present invention at the fertilized egg cell stage is preserved to be present in all of the germinal and somatic cells of the target mammal. The fact that the abnormal DNA of the present invention is present in the germinal cells of the animal after DNA transfection means that all of the offspring of the prepared animal have the abnormal DNA of the present invention in all of the germinal and somatic cells. Such an offspring that passaged the exogenous DNA of the present invention will have the abnormal DNA of the present invention in all of the germinal and somatic cells. A homozygous animal having the introduced DNA on both of homologous chromosomes can be acquired, and by crossing these male and female

animals, all the offspring can be bred to retain the DNA.

In a non-human mammal bearing the abnormal DNA of the present invention, the abnormal DNA of the present invention is overexpressed, and may eventually develop the function inactive type inadaptability to the protein of the present invention by inhibiting the function of endogenous normal DNA. Therefore, the animal can be utilized as a pathologic model animal for such a disease. For example, using the abnormal DNA transgenic animal of the present invention, it is possible to elucidate the pathological mechanism of the function inactive type inadaptability to the protein of the present invention and investigate how to treat this disease.

As a specific example of the availability, the transgenic animal overexpressing the abnormal DNA of the present invention is expected to serve as an experimental model to elucidate the mechanism of the functional inhibition (dominant negative effect) of a normal protein by the abnormal protein of the present invention in the function inactive type inadaptability of the protein of the present invention.

The mammal in which the abnormal exogenous DNA of the present invention has been transfected has a symptom of increase or decrease in the protein of the present invention in a free form and can thus be applied for example to a test for screening of a prophylactic/therapeutic agent for respiratory diseases [for example, chronic obstructive pulmonary disease (chronic bronchitis, pulmonary emphysema), diffuse panbronchiolitis, bronchial asthma, cystic fibrosis, hypersensitive pneumonia, pulmonary fibrosis etc.].

Other potential applications of two kinds of the DNA transgenic animals of the present invention described above further include:

(1) Use as a cell source for tissue culture;

(2) Elucidation of the relation to a peptide that is specifically expressed or activated by the protein of the present invention, by direct analysis of DNA or RNA in tissues of the DNA transgenic animal of the present invention or by analysis of the peptide tissues expressed by the DNA;

(3) Research on the function of cells derived from tissues that are usually cultured only with difficulty, using cells in tissues bearing the DNA cultured by a standard tissue culture technique;

(4) Screening of an agent that enhances the function of cells using the cells described in (3) above; and,

(5) Isolation and purification of the variant protein of the present invention and preparation of an antibody thereto; etc.

Furthermore, clinical conditions of a disease associated wit the protein of the

present invention, including the function inactive type inadaptability to the protein of the present invention can be determined by using the DNA transgenic animal of the present invention. Also, pathological findings on each organ in a disease model associated with the protein of the present invention can be obtained in more detail, leading to the development of a new method for treatment as well as the research and therapy of any secondary diseases associated with the disease.

It is also possible to obtain a free DNA-transfected cell by withdrawing each organ from the DNA transgenic animal of the present invention, mincing the organ and degrading with a proteinase such as trypsin, etc., followed by establishing the line of culturing or cultured cells. Furthermore, the DNA transgenic animal can serve to identify cells capable of producing the protein of the present invention, and to study in association with apoptosis, differentiation or propagation or on the mechanism of signal transduction in these properties to inspect any abnormality therein. Accordingly, the DNA transgenic animal can provide an effective research material for the protein of the present invention and for investigation of the function and effect thereof.

To develop a therapeutic agent for the treatment of diseases associated with the protein of the present invention, including the function inactive type inadaptability to the protein of the present invention, using the DNA transgenic animal of the present invention, an effective and rapid method for screening can be provided by using the method for inspection and the method for quantification, etc. described above. It is also possible to investigate and develop a method for DNA therapy for the treatment of diseases associated with the protein of the present invention, using the DNA transgenic animal of the present invention or a vector capable of expressing the exogenous DNA of the present invention.

(9) Knockout animal

The present invention provides a non-human mammal embryonic stem cell bearing the DNA of the present invention inactivated and a non-human mammal deficient in expressing the DNA of the present invention.

Thus, the present invention provides:

(1) A non-human mammal embryonic stem cell in which the DNA of the present invention is inactivated;

(2) The embryonic stem cell according to (1), wherein the DNA is inactivated by introducing a reporter gene (e.g., β-galactosidase gene derived from Escherichia coli);

(3) The embryonic stem cell according to (1), which is resistant to neomycin;

(4) The embryonic stem cell according to (1), wherein the non-human mammal is a rodent;

(5) The embryonic stem cell according to (4), wherein the rodent is mouse;

(6) A non-human mammal deficient in expressing the DNA of the present invention, wherein the DNA is inactivated;

(7) The non-human mammal according to (6), wherein the DNA is inactivated by inserting a reporter gene (e.g., β-galactosidase derived from Escherichia coli) therein and the reporter gene is capable of being expressed under control of a promoter for the DNA of the present invention;

(8) The non-human mammal according to (6), which is a rodent;

(9) The non-human mammal according to (8), wherein the rodent is mouse; and,

(10) A method of screening a compound that promotes or inhibits (preferably inhibits) the promoter activity to the DNA of the present invention, which comprises administering a test compound to the mammal of (7) and detecting expression of the reporter gene.

The non-human mammal embryonic stem cell in which the DNA of the present invention is inactivated refers to a non-human mammal embryonic stem cell that suppresses the ability of the non-human mammal to express the DNA by artificially mutating the DNA of the present invention, or the DNA has no substantial ability to express the protein of the present invention (hereinafter sometimes referred to as the knockout DNA of the present invention) by substantially inactivating the activities of the protein of the present invention encoded by the DNA (hereinafter merely referred to as ES cell).

As the non-human mammal, those described above are used.

Techniques for artificially mutating the DNA of the present invention include deletion of a part or all of the DNA sequence and insertion of or substitution with other DNA, by genetic engineering. By these variations, the knockout DNA of the present invention may be prepared, for example, by shifting the reading frame of a codon or by disrupting the function of a promoter or exon.

Specifically, the non-human mammal embryonic stem cell in which the DNA of the present invention is inactivated (hereinafter merely referred to as the ES cell with the DNA of the present invention inactivated or the knockout ES cell of the present invention) can be obtained by, for example, isolating the DNA of the present invention that the desired non-human mammal possesses, inserting a DNA fragment having a DNA sequence constructed by inserting a drug resistant gene such as a neomycin resistant gene

or a hygromycin resistant gene, or a reporter gene such as lacZ (β-galactosidase gene) or cat (chloramphenicol acetyltransferase gene), etc. into its exon site thereby to disable the functions of exon, or integrating to a chromosome of the target animal by, e.g., homologous recombination, a DNA sequence that terminates gene transcription (e.g., polyA additional signal, etc.) in the intron between exons, thus inhibiting the synthesis of complete messenger RNA and eventually destroying the gene (hereinafter simply referred to as a targeting vector). The thus-obtained ES cells to the southern hybridization analysis with a DNA sequence on or near the DNA of the present invention as a probe, or to PCR analysis with a DNA sequence on the targeting vector and another DNA sequence near the DNA of the present invention which is not included in the targeting vector as primers, to select the knockout ES cell of the present invention.

The parent ES cells to inactivate the DNA of the present invention by homologous recombination, etc. may be of a strain already established as described above, or may originally be established in accordance with a modification of the known method by Evans and Kaufman described above. For example, in the case of mouse ES cells, currently it is common practice to use ES cells of the 129 strain. However, since their immunological background is obscure, the C57BL/6 mouse or the BDF1 mouse (F1 between C57BL/6 and DBA/2), wherein the low ovum availability per C57BL/6 in the C57BL/6 mouse has been improved by crossing with DBA/2, may be preferably used, instead of obtaining a pure line of ES cells with the clear immunological genetic background and for other purposes. The BDF1 mouse is advantageous in that, when a pathologic model mouse is generated using ES cells obtained therefrom, the genetic background can be changed to that of the C57BL/6 mouse by back-crossing with the C57BL/6 mouse, since its background is of the C57BL/6 mouse, as well as being advantageous in that ovum availability per animal is high and ova are robust.

In establishing ES cells, blastocytes at 3.5 days after fertilization are commonly used. In addition thereto, embryos are preferably collected at the 8-cell stage, cultured until the blastocyte stage and then used thereby to efficiently obtain a large number of early stage embryos.

Although the ES cells used may be of either sex, male ES cells are generally more convenient for generation of a germ cell line chimera. It is also desirable that sexes are identified as soon as possible to save painstaking culture time.

Methods for sex identification of the ES cell include the method in which a gene in the sex-determining region on the Y-chromosome is amplified by the PCR process and detected. When this method is used, one colony of ES cells (about 50 cells) is sufficient

for sex-determination analysis, which karyotype analysis, for example G-banding method, requires about 106 cells; therefore, the first selection of ES cells at the early stage of culture can be based on sex identification, and male cells can be selected early, which saves a significant amount of time at the early stage of culture.

Also, second selection can be achieved by, for example, confirmation of the number of chromosomes by the G-banding method. It is usually desirable that the chromosome number of the obtained ES cells be 100% of the normal number. However, when it is difficult to obtain the cells having the normal number of chromosomes due to physical operations, etc. in the cell establishment, it is desirable that the ES cell is again cloned to a normal cell (e.g., in a mouse cell having the number of chromosomes being 2n = 40) after knockout of the gene of the ES cells.

Although the embryonic stem cell line thus obtained shows a very high growth potential, it must be subcultured with great care, since it tends to lose its ontogenic capability. For example, the embryonic stem cell line is cultured at about 37°C in a carbon dioxide incubator (preferably 5% carbon dioxide and 95% air, or 5% oxygen, 5% carbon dioxide and 90% air) in the presence of LIF (1 to 10000 U/ml) on appropriate feeder cells such as STO fibroblasts, treated with a trypsin/EDTA solution (normally 0.001 to 0.5% trypsin/0.1 to about 5 mM EDTA, preferably about 0.1% trypsin/1 mM EDTA) at the time of passage to obtain separate single cells, which are then plated on freshly prepared feeder cells. This passage is normally conducted every 1 to 3 days; it is desirable that cells be observed at the passage and cells found to be morphologically abnormal in culture, if any, be abandoned.

Where ES cells are allowed to reach a high density in mono-layers or to form cell aggregates in suspension under appropriate conditions, it is possible to differentiate the ES cells to various cell types, for example, pariental and visceral muscles, cardiac muscle or the like [M. J. Evans and M. H. Kaufman, Nature, 292, 154, 1981; G. R. Martin, Proc. Natl. Acad. Sci. U.S.A., 78, 7634, 1981; T. C. Doetschman et al., Journal of Embryology Experimental Morphology, 87, 27, 1985]. The cells deficient in expression of the DNA of the present invention, which are obtained from the differentiated ES cells of the present invention, are useful for cytological study of the protein of the present invention in vitro.

The non-human mammal deficient in expression of the DNA of the present invention can be identified from a normal animal by measuring the mRNA level in the subject animal by a publicly known method, and indirectly comparing the degrees of expression.

As the non-human mammal, those as given above are used.

With respect to the non-human mammal deficient in expression of the DNA of the present invention, the DNA of the present invention can be knockout by transfecting a targeting vector, prepared as described above, to mouse embryonic stem cells or mouse oocytes, and conducting homologous recombination in which a targeting vector DNA sequence, wherein the DNA of the present invention is inactivated by the transfection, is replaced with the DNA of the present invention on a chromosome of a mouse embryonic stem cell or mouse embryo.

The knockout cells with the disrupted DNA of the present invention can be identified by the southern hybridization analysis using as a probe a DNA fragment on or near the DNA of the present invention, or by the PCR analysis using as primers a DNA sequence on the targeting vector and another DNA sequence at the proximal region of other than the DNA of the present invention derived from mouse used in the targeting vector. When non-human mammal stem cells are used, a cell line wherein the DNA of the present invention is inactivated by homologous recombination is cloned; the resulting clones are injected to, e.g., a non-human mammalian embryo or blastocyst, at an appropriate stage such as the 8-cell stage. The resulting chimeric embryos are transplanted to the uterus of the pseudopregnant non-human mammal. The resulting animal is a chimeric animal constructed with both cells having the normal locus of the DNA of the present invention and those having an artificially mutated locus of the DNA of the present invention.

When some germ cells of the chimeric animal have a mutated locus of the DNA of the present invention, an individual, which entire tissue is composed of cells having a mutated locus of the DNA of the present invention can be selected from a series of offspring obtained by crossing between such a chimeric animal and a normal animal, e.g., by coat color identification, etc. The individuals thus obtained are normally deficient in heterozygous expression of the protein of the present invention. The individuals deficient in homozygous expression of the protein of the present invention can be obtained from offspring of the intercross between those deficient in heterozygous expression of the protein of the present invention.

When an oocyte is used, a DNA solution may be injected, e.g., into the prenucleus by microinjection thereby to obtain a transgenic non-human mammal having a targeting vector introduced in its chromosome. From such transgenic non-human mammals, those having a mutation at the locus of the DNA of the present invention can be obtained by selection based on homologous recombination.

As described above, the individuals in which the DNA of the present invention is knockout permit passage rearing under ordinary rearing conditions, after the individuals obtained by their crossing have proven to have been knockout.

Furthermore, the genital system may be obtained and retained by conventional methods. That is, by crossing male and female animals each having the inactivated DNA, homozygote animals having the inactivated DNA in both loci can be obtained. The homozygotes thus obtained may be reared so that one normal animal and two or more homozygotes are produced from a mother animal to efficiently obtain such homozygotes. By crossing male and female heterozygotes, homozygotes and heterozygotes having the inactivated DNA are proliferated and passaged.

The non-human mammal embryonic stem cell, in which the DNA of the present invention is inactivated, is very useful for preparing a non-human mammal deficient in expression of the DNA of the present invention.

Since the non-human mammal, in which the DNA of the present invention is inactivated, lacks various biological activities derived from the protein of the present invention, such an animal can be a disease model suspected of inactivated biological activities of the protein of the present invention and thus, offers an effective study to investigate the causes for and therapy for these diseases.

(9a) Method of screening the compound having therapeutic/prophylactic effects on diseases caused by deficiency, damages, etc. of the DNA of the present invention

The non-human mammal deficient in expression of the DNA of the present invention can be employed for screening the compound having therapeutic/prophylactic effects on diseases caused by deficiency, damages, etc. of the DNA of the present invention.

That is, the present invention provides a method of screening a compound or its salt having an effect of treating/preventing diseases caused by the deficiency or damage of the DNA of he present invention, for example respiratory diseases [for example, chronic obstructive pulmonary disease (chronic bronchitis, pulmonary emphysema), diffuse panbronchiolitis, bronchial asthma, cystic fibrosis, hypersensitive pneumonia, pulmonary fibrosis etc.], which comprises administering a test compound to a non-human mammal deficient in expression of the DNA of the present invention and observing/determining changes in the animal.

As the non-human mammal deficient in expression of the DNA of the present invention, which can be employed for the screening method, the same examples as

described above apply.

Examples of the test compound include peptides, proteins, non-peptide compounds, synthetic compounds, fermentation products, cell extracts, plant extracts, animal tissue extracts, blood plasma, etc. These compounds may be novel compounds or publicly known compounds.

Specifically, the non-human mammal deficient in expression of the DNA of the present invention is treated with a test compound, comparison is made with an intact animal for control and a change in each organ, tissue, disease conditions, etc. of the animal is used as an indicator to assess the therapeutic/prophylactic effects of the test compound.

For treating an animal to be tested with a test compound, for example, oral administration, intravenous injection, etc. are applied, and the treatment can be appropriately selected depending on conditions of the test animal, properties of the test compound, etc. Furthermore, a dose of the test compound to be administered can be appropriately chosen depending on the administration route, property of the test compound, etc.

For example, when a compound having a prophylactic/therapeutic effect on respiratory diseases [for example, chronic obstructive pulmonary disease (chronic bronchitis, pulmonary emphysema), diffuse panbronchiolitis, bronchial asthma, cystic fibrosis, hypersensitive pneumonia, pulmonary fibrosis etc.] is screened, a test compound is administered to the non-human mammal deficient in expression of the DNA according to the present invention, and the tissue is observed with time for a difference in emphysema of the lung from that of a group not given the test compound.

In the screening method, when a test compound is administered to a test animal and the disease conditions of the test animal are improved by at least about 10%, preferably at least about 30% and more preferably at least about 50%, the test compound can be selected as the compound having therapeutic/prophylactic effects on the diseases described above.

The compound obtained using the above screening method is a compound selected from the test compounds described above and exhibits therapeutic/prophylactic effects on diseases caused by deficiencies, damages, etc. of the protein of the present invention. Therefore, the compound can be employed as a safe and low toxic prophylactic/ therapeutic agents for these diseases. Furthermore, compounds derived from the compound obtained by the screening described above may also be used as well.

The compound obtained by the screening method above may form salts, and

may be used in the form of salts with physiologically acceptable acids (e.g., inorganic acids, organic acids, etc.) or bases (e.g., alkali metal salts), preferably in the form of physiologically acceptable acid addition salts. Examples of such salts are salts with inorganic acids (e.g., hydrochloric acid, phosphoric acid, hydrobromic acid, sulfuric acid, etc.), salts with organic acids (e.g., acetic acid, formic acid, propionic acid, fumaric acid, maleic acid, succinic acid, tartaric acid, citric acid, malic acid, oxalic acid, benzoic acid, methanesulfonic acid, benzenesulfonic acid, etc.) and the like.

A pharmaceutical comprising the compound obtained by the above screening method or salts thereof can be manufactured in a manner similar to the method for preparing the pharmaceutical comprising the protein of the present invention described hereinabove.

Since the pharmaceutical preparation thus obtained is safe and low toxic, it can be administered to human or mammal (e.g., rat, mouse, guinea pig, rabbit, ovine, swine, bovine, equine, feline, canine, simian, etc.).

The dose of the compound or its salt may vary depending upon target disease, subject to be administered, route of administration, etc. For example, when the compound is orally administered, the compound is administered to the adult patient with pulmonary emphysema (as 60 kg body weight) generally in a dose of about 0.1 to 100 mg, preferably about 1.0 to 50 mg and more preferably about 1.0 to 20 mg. In parenteral administration, a single dose of the compound may vary depending upon subject to be administered, target disease, etc. When the compound is administered to the adult patient with breast cancer (as 60 kg body weight) in the form of an injectable preparation, it is advantageous to administer the compound in a single dose of about 0.01 to about 30 mg, preferably about 0.1 to about 20 mg and more preferably about 0.1 to about 10 mg a day. For other animal species, the corresponding dose as converted per 60 kg weight can be administered.

(9b) Method of screening a compound that promotes or inhibits the activity of a promoter to the DNA of the present invention

The present invention provides a method of screening a compound or its salts that promote or inhibit the activity of a promoter to the DNA of the present invention, which comprises administering a test compound to a non-human mammal deficient in expression of the DNA of the present invention and detecting the expression of a reporter gene.

In the screening method described above, an animal in which the DNA of the

present invention is inactivated by introducing a reporter gene and the reporter gene is expressed under control of a promoter to the DNA of the present invention is used as the non-human mammal deficient in expression of the DNA of the present invention, which is selected from the aforesaid non-human mammals deficient in expression of the DNA of the present invention.

The test compounds are those as given above.

As the reporter gene, the same specific examples apply to this screening method. Preferably, there are used β-galactosidase (lacZ), soluble alkaline phosphatase gene, luciferase gene and the like.

Since the reporter gene is present under control of a promoter to the DNA of the present invention in the non-human mammal deficient in expression of the DNA of the present invention wherein the DNA of the present invention is substituted with the reporter gene, the activity of the promoter can be detected by tracing the expression of a substance encoded by the reporter gene.

When a part of the DNA region encoding the protein of the present invention is substituted with, e.g., β-galactosidase gene (lacZ) derived from Escherichia coli, β-galactosidase is expressed in a tissue where the protein of the present invention should originally be expressed, instead of the protein of the present invention. Thus, the state of expression of the protein of the present invention can be readily observed in vivo of an animal by staining with a reagent, e.g., 5-bromo-4-chloro-3-indolyl-β-galactopyranoside (X-gal) which is substrate for β-galactosidase. Specifically, a mouse deficient in the protein of the present invention, or its tissue section is fixed with glutaraldehyde, etc. After washing with phosphate buffered saline (PBS), the system is reacted with a staining solution containing X-gal at room temperature or about 37°C for approximately 30 minutes to an hour. After the β-galactosidase reaction is terminated by washing the tissue preparation with 1 mM EDTA/PBS solution, the color formed is observed. Alternatively, mRNA encoding lacZ may be detected in a conventional manner.

The compound or salts thereof obtained using the screening method described above are compounds that are selected from the test compounds described above and that promote or inhibit the promoter activity to the DNA of the present invention.

The compound obtained by the screening method above may form salts, and may be used in the form of salts with physiologically acceptable acids (e.g., inorganic acids, etc.) or bases (e.g., alkali metals, etc.) or the like, especially in the form of physiologically acceptable acid addition salts. Examples of such salts are salts with inorganic acids (e.g., hydrochloric acid, phosphoric acid, hydrobromic acid, sulfuric acid,

etc.), salts with organic acids (e.g., acetic acid, formic acid, propionic acid, fumaric acid, maleic acid, succinic acid, tartaric acid, citric acid, malic acid, oxalic acid, benzoic acid, methanesulfonic acid, benzenesulfonic acid, etc.) and the like.

The compound or its salt promoting or inhibiting the promoter activity to the DNA of the present invention can regulate the expression of the protein of the present invention and can regulate the functions of the said protein, and can thus be used for example as a prophylactic/therapeutic agent for diseases in respiratory diseases [for example, chronic obstructive pulmonary disease (chronic bronchitis, pulmonary emphysema), diffuse panbronchiolitis, bronchial asthma, cystic fibrosis, hypersensitive pneumonia, pulmonary fibrosis etc.], preferably as a prophylactic/therapeutic agent for chronic obstructive pulmonary disease etc.

In addition, compounds derived from the compound obtained by the screening described above may also be used as well.

A pharmaceutical comprising the compound obtained by the above screening method or a salt thereof can be manufactured in a manner similar to the method for preparing the pharmaceutical comprising the protein of the present invention described above.

Since the pharmaceutical preparation thus obtained is safe and low toxic, it can be administered to human or mammal (e.g., rat, mouse, guinea pig, rabbit, ovine, swine, bovine, equine, feline, canine, simian, etc.).

A dose of the compound or its salt may vary depending on target disease, subject to be administered, route for administration, etc.; when the compound that inhibits the promoter activity to the DNA encoding the protein A of the present invention is orally administered, the compound is administered to the adult patient with pulmonary emphysema (as 60 kg body weight) normally in a daily dose of about 0.1 to 100 mg, preferably about 1.0 to 50 mg and more preferably about 1.0 to 20 mg. In parenteral administration, a single dose of the compound may vary depending on subject to be administered, target disease, etc. but when the compound of inhibiting the promoter activity to the DNA of the present invention is administered to the adult patient with pulmonary emphysema (as 60 kg body weight) in the form of injectable preparation, it is advantageous to administer the compound intravenously to the patient in a daily dose of about 0.01 to about 30 mg, preferably about 0.1 to about 20 mg and more preferably about 0.1 to about 10 mg. For other animal species, the corresponding dose as converted per 60 kg weight can be administered.

As stated above, the non-human mammal deficient in expression of the DNA of

the present invention is extremely useful for screening the compound or its salt that promotes or inhibits the promoter activity to the DNA of the present invention and, can greatly contribute to elucidation of causes for various diseases suspected of deficiency in expression of the DNA of the present invention and for the development of prophylactic/therapeutic agents for these diseases.

In addition, a so-called transgenic animal (gene transferred animal) can be prepared by using a DNA containing the promoter region of the protein of the present invention, ligating genes encoding various proteins at the downstream and injecting the same into oocyte of an animal. It is thus possible to synthesize the protein therein specifically and study its activity in vivo. When an appropriate reporter gene is ligated to the promoter site described above and a cell line that expresses the gene is established, the resulting system can be utilized as the search system for a low molecular compound having the action of specifically promoting or inhibiting the in vivo productivity of the protein itself of the present invention.

In the specification, where bases, amino acids, etc. are expressed in abbreviations, they are denoted by abbreviations in accordance with the IUPAC-IUB Commission on Biochemical Nomenclature or by conventional abbreviations in the art, examples of which are shown below. For amino acids that may have the optical isomer, L form is presented unless otherwise indicated.

DNA	: deoxyribonucleic acid
cDNA	: complementary deoxyribonucleic acid
A	: adenine
T	: thymine
G	: guanine
C	: cytosine
RNA	: ribonucleic acid
mRNA	: messenger ribonucleic acid
dATP	: deoxyadenosine triphosphate
dTTP	: deoxythymidine triphosphate
dGTP	: deoxyguanosine triphosphate
dCTP	: deoxycytidine triphosphate
ATP	: adenosine triphosphate
EDTA	: ethylenediaminetetraacetic acid

SDS : sodium dodecyl sulfate
Gly : glycine
Ala : alanine
Val : valine
Leu : leucine
Ile : isoleucine
Ser : serine
Thr : threonine
Cys : cysteine
Met : methionine
Glu : glutamic acid
Asp : aspartic acid
Lys : lysine
Arg : arginine
His : histidine
Phe : phenylalanine
Tyr : tyrosine
Trp : tryptophan
Pro : proline
Asn : asparagine
Gln : glutamine
pGlu : pyroglutamic acid
Sec : selenocysteine

Substituents, protecting groups and reagents generally used in this specification are presented as the codes below.

Me : methyl group
Et : ethyl group
Bu : butyl group
Ph : phenyl group
TC : thiazolidine-4(R)-carboxamido group
Tos : p-toluenesulfonyl
CHO : formyl
Bzl : benzyl

Cl2-Bzl : 2,6-dichlorobenzyl
Bom : benzyloxymethyl
Z : benzyloxycarbonyl
Cl-Z : 2-chlorobenzyloxycarbonyl
Br-Z : 2-bromobenzyl oxycarbonyl
Boc : t-butoxycarbonyl
DNP : dinitrophenol
Trt : trityl
Bum : t-butoxymethyl
Fmoc : N-9-fluorenyl methoxycarbonyl
HOBt : 1-hydroxybenztriazole
HOOBt : 3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine
HONB : 1-hydroxy-5-norbornene-2,3-dicarboxyimide
DCC : N,N'-dicyclohexylcarbodiimide

The sequence identification numbers in the sequence listing of the specification indicates the following sequence.

[SEQ ID NO: 1]

This shows the base sequence of CH25H.

[SEQ ID NO: 2]

This shows the amino acid sequence of a protein translated from the base sequence represented by SEQ ID NO: 1.

[SEQ ID NO: 3]

This shows the base sequence of PLAB.

[SEQ ID NO: 4]

This shows the amino acid sequence of a protein translated from the base sequence represented by SEQ ID NO: 3.

[SEQ ID NO: 5]

This shows the base sequence of CSF3.

[SEQ ID NO: 6]

This shows the amino acid sequence of a protein translated from the base sequence represented by SEQ ID NO: 5.

[SEQ ID NO: 7]

This shows the base sequence of RHO6.

[SEQ ID NO: 8]

This shows the amino acid sequence of a protein translated from the base sequence represented by SEQ ID NO: 7.

[SEQ ID NO: 9]

This shows the base sequence of SFN.

[SEQ ID NO: 10]

This shows the amino acid sequence of a protein translated from the base sequence represented by SEQ ID NO: 9.

[SEQ ID NO: 11]

This shows the base sequence of SSB1.

[SEQ ID NO: 12]

This shows the amino acid sequence of a protein translated from the base sequence represented by SEQ ID NO: 11.

[SEQ ID NO: 13]

This shows the base sequence of TNFAIP3.

[SEQ ID NO: 14]

This shows the amino acid sequence of a protein translated from the base sequence represented by SEQ ID NO: 13.

[SEQ ID NO: 15]

This shows the base sequence of TNFAIP6.

[SEQ ID NO: 16]

This shows the amino acid sequence of a protein translated from the base sequence represented by SEQ ID NO: 15.

[SEQ ID NO: 17]

This shows the base sequence of IER3.

[SEQ ID NO: 18]

This shows the amino acid sequence of a protein translated from the base sequence represented by SEQ ID NO: 17.

[SEQ ID NO: 19]

This shows the base sequence of GADD45A.

[SEQ ID NO: 20]

This shows the amino acid sequence of a protein translated from the base sequence represented by SEQ ID NO: 21.

[SEQ ID NO: 21]

This shows the base sequence of GADD45B.

[SEQ ID NO: 22]

This shows the amino acid sequence of a protein translated from the base sequence represented by SEQ ID NO: 21.

[SEQ ID NO: 23]

This shows the base sequence of 1L1RN.

[SEQ ID NO: 24]

This shows the amino acid sequence of a protein translated from the base sequence represented by SEQ ID NO: 23.

[SEQ ID NO: 25]

This shows the base sequence of SOCS2.

[SEQ ID NO: 26]

This shows the amino acid sequence of a protein translated from the base sequence represented by SEQ ID NO: 25.

[SEQ ID NO: 27]

This shows the base sequence of SOCS3.

[SEQ ID NO: 28]

This shows the amino acid sequence of a protein translated from the base sequence represented by SEQ ID NO: 27.

[SEQ ID NO: 29]

This shows the base sequence of MMP19.

[SEQ ID NO: 30]

This shows the amino acid sequence of a protein translated from the base sequence represented by SEQ ID NO: 29.

[SEQ ID NO: 31]

This shows the base sequence of DUSP2.

[SEQ ID NO: 32]

This shows the amino acid sequence of a protein translated from the base sequence represented by SEQ ID NO: 31.

[SEQ ID NO: 33]

This shows the base sequence of DUSP5.

[SEQ ID NO: 34]

This shows the amino acid sequence of a protein translated from the base sequence represented by SEQ ID NO: 33.

[SEQ ID NO: 35]

This shows the base sequence of STC1.

[SEQ ID NO: 36]

This shows the amino acid sequence of a protein translated from the base sequence represented by SEQ ID NO: 35.

[SEQ ID NO: 37]

This shows the base sequence of LDLR.

[SEQ ID NO: 38]

This shows the amino acid sequence of a protein translated from the base sequence represented by SEQ ID NO: 37.

[SEQ ID NO: 39]

This shows the base sequence of TNFRSF10B.

[SEQ ID NO: 40]

This shows the amino acid sequence of a protein translated from the base sequence represented by SEQ ID NO: 39.

[SEQ ID NO: 41]

This shows the base sequence of TNFRSF12A.

[SEQ ID NO: 42]

This shows the amino acid sequence of a protein translated from the base sequence represented by SEQ ID NO: 41.

[SEQ ID NO: 43]

This shows the base sequence of MAP3K8.

[SEQ ID NO: 44]

This shows the amino acid sequence of a protein translated from the base sequence represented by SEQ ID NO: 43.

[SEQ ID NO: 45]

This shows the base sequence of EGR1.

[SEQ ID NO: 46]

This shows the amino acid sequence of a protein translated from the base sequence represented by SEQ ID NO: 45.

[SEQ ID NO: 47]

This shows the base sequence of EGR3.

[SEQ ID NO: 48]

This shows the amino acid sequence of a protein translated from the base sequence represented by SEQ ID NO: 47.

[SEQ ID NO: 49]

This shows the base sequence of ADAMTS1.

[SEQ ID NO: 50]

This shows the amino acid sequence of a protein translated from the base sequence represented by SEQ ID NO: 49.

[SEQ ID NO: 51]

This shows the base sequence of TFPI2.

[SEQ ID NO: 52]

This shows the amino acid sequence of a protein translated from the base sequence represented by SEQ ID NO: 51.

[SEQ ID NO: 53]

This shows the base sequence of OSM.

[SEQ ID NO: 54]

This shows the amino acid sequence of a protein translated from the base sequence represented by SEQ ID NO: 53.

[SEQ ID NO: 55]

This shows the base sequence of TNC.

[SEQ ID NO: 56]

This shows the amino acid sequence of a protein translated from the base sequence represented by SEQ ID NO: 55.

[SEQ ID NO: 57]

This shows the base sequence of EDG3.

[SEQ ID NO: 58]

This shows the amino acid sequence of a protein translated from the base sequence represented by SEQ ID NO: 57.

[SEQ ID NO: 59]

This shows the base sequence of GPR73L1.

[SEQ ID NO: 60]

This shows the amino acid sequence of a protein translated from the base sequence represented by SEQ ID NO: 59.

[SEQ ID NO: 61]

This shows the base sequence of SFRP2.

[SEQ ID NO: 62]

This shows the amino acid sequence of a protein translated from the base sequence represented by SEQ ID NO: 61.

[SEQ ID NO: 63]

This shows the base sequence of HIMAP2.

[SEQ ID NO: 64]

This shows the amino acid sequence of a protein translated from the base sequence represented by SEQ ID NO: 63.

[SEQ ID NO: 65]

This shows the base sequence of SSTR1.

[SEQ ID NO: 66]

This shows the amino acid sequence of a protein translated from the base sequence represented by SEQ ID NO: 65.

[SEQ ID NO: 67]

This shows the base sequence of primer 1 used in detection of the expression level of CH25H gene.

[SEQ ID NO: 68]

This shows the base sequence of primer 2 used in detection of the expression level of CH25H gene.

[SEQ ID NO: 69]

This shows the base sequence of primer 3 used in EXAMPLE 5.

[SEQ ID NO: 70]

This shows the base sequence of primer 4 used in EXAMPLE 5.

[SEQ ID NO: 71]

This shows the base sequence of primer 5 used in EXAMPLE 5.

[SEQ ID NO: 72]

This shows the base sequence of primer 6 used in EXAMPLE 5.

[SEQ ID NO: 73]

This shows the amino acid sequence of the antigenic peptide used in EXAMPLE 5.

EXAMPLES

Hereinafter the present invention will be described more specifically by referring to EXAMPLES but is not deemed to be limited thereto.

EXAMPLE 1

(1) Acquisition of excised lung samples from lung cancer patients with a complication of COPD

From lung cancer patients required to undergo lung excision, lung samples removed in a lung excision operation were provided as materials for study. For acquisition of the lung samples, we obtained an approval of Ethical Committee in

Tohoku University and an informed consent from the patients.

In diagnosis of COPD, we measured patients' vital capacity (VC (L)), % lung capacity (%VC), forced vital capacity (FVC (L)), % forced vital capacity (%FVC), forced expiratory volume in 1 second (FEV1 (L)), % forced expiratory volume in 1 second (%FEV1), one-second forced expiratory volume rate (%) (FEV1/FVC (%)), total lung capacity (TLC (L)), % total lung capacity (%TLC), functional residual capacity (FRC (L)), % functional residual capacity (%FRC), residual capacity (RC (L)), % residual volume (%RV), residual ratio (RV/TLC (%)), Diffusing capacity of the lung for carbon monoxide (DLCO), % Diffusing capacity of the lung for carbon monoxide (%DLCO), alveolar ventilation (DLCO/VA), arterial blood oxygen partial pressure (PaO_2), arterial blood carbon dioxide partial pressure ($PaCO_2$), bicarbonate ion (HCO_3), and functional residual ratio (FRC/TLC (%)), and a patient showing $FEV_1/FVC < 70\%$ and $\%FEV_1$ □ 80% was diagnosed as a light case (stage I) COPD, a patient showing $FEV_1/FVC < 70\%$ and $50\% < \%FEV_1 < 80\%$ was diagnosed as a moderate case (stage IIA) COPD, and a patient showing $FEV_1/FVC < 70\%$ and $30\% < \%FEV_1 < 50\%$ was diagnosed as a moderate case (stage IIB) COPD.

A patient with lung cancer showing $FEV_1/FVC \geq 70\%$ and free from symptoms such as chronic cough and phlegm was diagnosed as non-COPD.

Further, smoking history was investigated, and a patient without smoking history was classified into non-smoker, a patient with smoking history in the past was classified into ex-smoker, and a patient who smokes even at present was classified into smoker.

The patients with lung cancer were classified into a non-COPD and no-smoker group (NN group, 12 cases), a non-COPD and ex-smoker group (NE group, 6 cases), a non-COPD and smoker group (NS group, 5 cases), a stage I COPD group (CE1 group, 7 cases), a stage IIA COPD group (CE2A group, 6 cases) and a stage IIB COPD group (CE2B group, 2 cases).

(2) Searching for genes whose expression fluctuated in lung tissues of COPD patients

To reveal genes whose expression fluctuated specifically in lung tissues of COPD patients, lung tissue samples after the operation of removing the lungs from the lung cancer patients with a complication of COPD were frozen in liquid nitrogen, then milled with a frozen-tissue milling device, and immersed in Isogen (Nippon Gene) in a 10-fold excess amount relative to the wet lungs, to prepare total RNAs according to its attached protocol. Among all samples from which total RNAs were prepared, total

RNAs from the NN group (5 cases), NE group (3 cases), NS group (2 cases), CE1 group (3 cases), and CE2A group (2 cases) were used as materials, and the 15 samples in total were used in gene expression analysis with an oligonucleotide microarray (Human Genome U133A, U133B; Affymetrix). The experimental method was in accordance with an experimental guide (Expression analysis technical manual) from Affymetrix.

The expression level of each gene was expressed as expression level assuming that the central value of expression levels of all genes in each oligonucleotide microarray was 1, and the mean among the groups was determined and compared as the gene expression value in each group.

The fluctuation of gene expression (COPD/non) in COPD was calculated according to the following equation, and the results are shown in Tables 1, 2 and 3.

$$\text{COPD/non} = \frac{\dfrac{(\text{CE1 group (3 cases) gene expression value} + \text{CE2A group (2 cases) gene expression value})}{5}}{\dfrac{(\text{NN group (5 cases) gene expression value} + \text{NE group (3 cases) gene expression value} + \text{NS group (2 cases) gene expression value})}{10}}$$

Table 1

Symbol of gene	Name of gene	NN	NE	NS	CE1	CE2A	COPD/non
CH25H	cholesterol 25-hydroxylase	0.58	0.67	1.15	1.71	3.16	3.2
PLAB	prostate differentiation factor(=GDF15)	1.27	1.05	1.92	5.19	6.90	4.4
CSF3	colony stimulating factor 3 (granulocyte)	0.12	0.02	0.54	4.22	1.46	17.9
RHO6	GTP-binding protein(=Rho6, Socius)	0.54	0.22	1.10	6.09	2.90	8.6
SFN	stratifin	0.47	0.61	0.97	5.57	2.82	7.3
SSB1	SPRY domain-containing SOCS box protein SSB-1	0.43	0.36	0.46	2.02	1.71	4.6
TNFAIP3	tumor necrosis factor, alpha-induced protein 3	0.91	0.98	2.36	5.08	5.80	4.4
TNFAIP6	tumor necrosis factor, alpha-induced protein 6	0.19	0.29	0.73	1.02	1.76	4.0
IER3	immediate early response 3	4.00	6.59	11.53	27.43	26.59	4.3
GADD45A	growth arrest and DNA-damage-inducible, alpha	0.80	0.96	1.54	3.54	3.99	3.7
GADD45B	growth arrest and DNA-damage-inducible, beta	2.61	3.02	5.62	13.79	7.83	3.4
IL1RN	interleukin 1 receptor antagonist	0.90	1.00	1.61	5.05	1.91	3.5
SOCS2	suppressor of cytokine signaling 2	0.88	0.70	1.45	3.42	2.33	3.2
SOCS3	suppressor of cytokine signaling 3	0.54	0.67	1.64	2.94	2.37	3.4
MMP19	matrix metalloproteinase 19	0.81	0.86	0.69	3.32	1.82	3.4

Table 2

DUSP2	dual specificity phosphatase 2	0.16	0.37	0.90	1.51	0.81	3.3
DUSP5	dual specificity phosphatase 5	1.04	1.08	3.91	4.73	3.40	2.6
STC1	stanniocalcin 1	0.19	0.26	0.30	0.82	0.66	3.2
LDLR	low density lipoprotein receptor (familial hypercholesterolemia)	3.88	2.83	7.28	15.77	8.80	3.1
TNFRSF10B	tumor necrosis factor receptor superfamily, member 10b	0.74	0.79	1.30	2.39	1.71	2.4
TNFRSF12A	tumor necrosis factor receptor superfamily, member 12A	1.75	1.56	2.14	5.39	4.89	2.9
MAP3K8	mitogen-activated protein kinase kinase kinase 8	0.21	0.20	0.29	0.56	0.64	2.6
EGR1	early growth response 1	2.96	2.66	4.52	6.34	6.24	2.0
EGR3	early growth response 3	0.39	0.81	1.15	1.42	2.36	2.7
ADAMTS1	a disintegrin-like and metalloprotease (reprolysin type) with thrombospondin type 1 motif, 1	1.21	1.60	4.40	3.92	3.26	1.9
TFPI2	tissue factor pathway inhibitor 2	0.34	1.81	0.65	0.93	2.22	1.7
OSM	oncostatin M	0.33	0.43	0.97	1.65	1.04	2.89
TNC	ESTs	0.25	0.31	0.65	0.73	1.02	2.43
EDG3	endothelial differentiation, sphingolipid G-protein-coupled receptor, 3	0.20	0.22	0.50	0.40	0.82	2.15
GPR73L1	G protein-coupled receptor 73-like 1	0.20	0.30	0.41	0.51	0.60	2.01
SFRP2	secreted frizzled-related protein 2	1.43	9.16	13.52	6.87	16.04	1.71

Table 3

Symbol of gene	Name of gene	NN	NE	NS	CE1	CE2A	COPD/non
HIMAP2	DKFZP586D0824 protein	0.95	0.93	0.85	0.40	0.39	0.43
SSTR1	somatostatin receptor 1	0.73	0.61	0.47	0.17	0.33	0.36

As a result, CH25H (NH_003956) (SEQ ID NO: 1), PLAB (AF003934) (SEQ ID NO: 3), CSF3 (NM_000759) (SEQ ID NO: 5), RH06 (NM_014470) (SEQ ID NO: 7), SFN (BC000329) (SEQ ID NO: 9), SSB1 (NM_25106) (SEQ ID NO: 11), TNFAIP3 (NM_006290) (SEQ ID NO: 13), TNFAIP6 (NM_007115) (SEQ ID: 15), IER3 (NM_003897) (SEQ ID NO: 17), GADD45A (NM_001924) (SEQ ID NO: 19), GADD45B (AF087853) (SEQ ID NO: 21), IL1RN (NM_173841) (SEQ ID NO: 23), SOCS2 (NM_003877) (SEQ ID NO: 25), SOCS3 (NM_003955) (SEQ ID NO: 27), MMP19 (U38321) (SEQ ID NO: 29), DUSP2 (NM_004418) (SEQ ID NO: 31), DUSP5 (U16996) (SEQ ID NO: 33), STC1 (U46768) (SEQ ID NO: 35), LDLR (NM_000527) (SEQ ID NO: 37), TNFRSF10B (AF016266) (SEQ ID NO: 39), TNFRSF12A (NM_016639) (SEQ ID NO: 41), MAP3K8 (NM_005204) (SEQ ID NO: 43), EGR1 (NM_001964) (SEQ ID NO: 45), EGR3 (NM_004430) (SEQ ID NO: 47), ADAMTS1 (NM_006988) (SEQ ID NO: 49), TFPI2 (L27624) (SEQ ID NO: 51), OSM (NM_020530) (SEQ ID NO: 53), TNC (NM_002160) (SEQ ID NO: 55), EDG3 (NM_005226) (SEQ ID NO: 57), GPR73L1 (NM_144773) (SEQ ID NO: 59) and SFRP2 (AY359001) (SEQ ID NO: 61) were detected as genes whose expression was increased as the morbid state of COPD proceeded (Tables 1 and 2).

HIMAP2 (NM_015660) (SEQ ID NO: 63) and SSTR1 (NM_001049) (SEQ ID NO: 65) were detected as genes whose expression was decreased as the morbid state of COPD proceeded (Table 3).

EXAMPLE 2

Analysis of correlation between expression fluctuation and respiratory functions

To determine whether the expression of the genes whose expression was observed to fluctuate in EXAMPLE 1 was related to the morbid state of COPD, the correlation between the expression level of each gene and respiratory functions (% forced expiratory volume in 1 second, % CO lung diffusing capacity) was analyzed.

It was found that the expression of CH25H (SEQ ID NO: 1), PLAB (SEQ ID NO: 3), CSF3 (SEQ ID NO: 5), RH06 (SEQ ID NO: 7), SFN (SEQ ID NO: 9), SSB1 (SEQ ID NO: 11), TNFAIP3 (SEQ ID NO: 13), TNFAIP6 (SEQ ID: 15), IER3 (SEQ ID NO: 17), GADD45A (SEQ ID NO: 19), GADD45B (SEQ ID NO: 21), IL1RN (SEQ ID NO: 23), SOCS2 (SEQ ID NO: 25), SOCS3 (SEQ ID NO: 27), MMP19 (SEQ ID NO: 29), DUSP2 (SEQ ID NO: 31), DUSP5 (SEQ ID NO: 33), STC1 (SEQ ID NO: 35), LDLR (SEQ ID NO: 37), TNFRSF10B (SEQ ID NO: 39), TNFRSF12A (SEQ ID NO: 41), MAP3K8 (SEQ ID NO: 43), EGR1 (SEQ ID NO:

45), EGR3 (SEQ ID NO: 47), ADAMTS1 (SEQ ID NO: 49), TFPI2 (SEQ ID NO: 51), OSM (SEQ ID NO: 53), TNC (SEQ ID NO: 55), EDG3 (SEQ ID NO: 57), GPR73L1 (SEQ ID NO: 59), SFRP2 (SEQ ID NO: 61), HIMAP2 (SEQ ID NO: 63) and SSTR1 (SEQ ID NO: 65) was correlated with the respiratory functions (% forced expiratory volume in 1 second, % CO lung diffusing capacity).

EXAMPLE 3

(1) Confirmation of fluctuation in expression of CH25H gene by quantitative RT-PCR method

The fluctuation in expression of CH25H (SEQ ID NO: 1) was examined by the quantitative RT-PCR method using the whole lung excised samples [NN group (12 cases), NE group (6 cases), NS group (5 cases), CE1 group (7 cases), CE2A group (6 cases)].

Using 500 ng total RNA prepared in EXAMPLE 1 as the starting material, cDNA was synthesized by reverse transcription reaction in 50 μl reaction solution with TaqMan Gold RT-PCR Kit (Applied Biosystems). The reaction solution was diluted 2.5-fold with distilled water, and 2 μl of the dilution was used in real-time quantitative PCR method using ABI PRISM 7900 sequence detection system (Applied Biosystems) and QuantiTect SYBR Green PCR Kit (QIAGEN) to determine the Ct value of each gene. The primers used in the quantitative PCR were designed using Primer Express program (Applied Biosystems) [primer 1 (SEQ ID NO: 67), primer 2 (SEQ ID NO: 68)]. The Ct value of GAPDH gene as house-keeping gene was determined in an analogous manner using TaqMan GAPDH control reagents (Applied Biosystems), and by the ΔCT method, the expression level of CH25H gene per GAPDH gene was determined, and expression per individual among the groups was compared (Fig. 1). Further, the correlation between the gene expression levels of the total samples [NN group (12 cases), NE group (6 cases), NS group (5 cases), CE1 group (7 cases), CE2A group (6 cases)] and the respiratory functions (% forced expiratory volume in 1 second, % CO lung diffusing capacity) was analyzed (Figs. 2 and 3).

It was thereby confirmed that the expression of CH25H gene (SEQ ID NO: 1) was increased in the lungs of the COPD patients.

CH25H is a kind of cholesterol hydroxylase. Accordingly, how the expression of each of CYP3A4, CYP7A1, CYP46 and CYP27A1, that is, cholesterol hydroxylases other than CH25H, was changed in the COPD patients was examined

by extracting and comparing the expression values of the respective genes from the GeneChip data shown in EXAMPLE 1. As a result, the expression of each of CYP3A4, CYP7A1, CYP46 and CYP27A1 did not fluctuate in the COPD patients. It was thus found that the cholesterol hydroxylase whose expression fluctuated as the morbid state of COPD proceeded was only CH25H.

(2) Distribution of CH25H gene in tissues

The distribution of CH25H, CYP3A4, CYP7A1, CYP46 and CYP27A1 in tissues was examined by using 2 μl Human MTC Panel I, Human MTC Panel II, Human Immune System MTC Panel, Human Blood Fractions MTC Panel (all of which are manufactured by Clontech), and the gene expression levels therein were measured according to the quantitative PCR shown in EXAMPLE 1. As each probe, the corresponding probe was selected from Assays on demand gene expression product (Applied Biosystems) and used.

As a result, CH25H had been expressed specifically in the lung. Except for CH25H, only CYP27A1 had been expressed at high level in the lung.

From the foregoing, it is estimated that CH25H is a sole cholesterol hydroxylase participating in the morbid state of COPD.

EXAMPLE 4

Analysis of fluctuation in CH25H gene expression in COPD model mouse

To examine the involvement of CH25H in the morbid state of COPD, the analysis of functions of CH25H was conducted by using a cigarette smoke-exposed mouse reflecting the morbid state of COPD (Toxicological Science vol. 51, pp. 289-299, 1999).

First, a fluctuation in expression of mouse CH25H gene in a pulmonary/bronchial lavage fluid of the mouse exposed to cigarette smoke was examined.

The mouse exposed to cigarette smoke was created by exposing a C57BL/6 mouse (7-week-old, male) to cigarette smoke under the following conditions. As the cigarette smoke, 3% diluted smoke of Kentucky reference cigarette 2R4F from which a filter had been cut off was used, and the mouse was exposed daily to 150 puffs/15 min → interval/15 min → 150 puffs/15 min → interval/15 min → 150 puffs/15 min → interval/15 min →150 puffs/15 min (40 cigarettes). After exposure for 2 or 3 days, the lung was excised, and according to the method shown in

EXAMPLE 1, total RNA was prepared. Subsequently, the expression levels of mouse CH25H gene and mouse CYP27A1 gene were measured according to the real-time quantitative PCR method shown in EXAMPLE 1. A tracheal canula was introduced into the mouse under anesthesia with pentobarbital after exposure to cigarette smoke for 3 days, and 0.5 ml PBS was injected 3 times into the lung and recovered the fluid therefrom. A fluctuation in the expression of mouse CH25H and mouse CYP27A1 in inflammatory cells in the bronchoalveolar lavage fluid was also analyzed. As each probe, the corresponding probe was selected from Assays on demand gene expression product (Applied Biosystems) and used. The expression level of each gene was calculated as relative expression value to rodent GAPDH by the comparative Ct value method.

As a result, the expression of the mouse CH25H gene was increased in the pulmonary/bronchoalveolar lavage fluid by exposure to cigarette smoke (Fig. 4A, B). On the other hand, the gene expression of CYP27A1 which is another cholesterol hydroxylase known to be expressed in the lung was reduced by exposure to cigarette smoke (Fig. 4A, B).

From these results, it was found that the expression of CH25H is increased in the cigarette smoke-exposed model reflecting the morbid state of COPD.

EXAMPLE 5

Fluctuation in the amount of 25-hydroxycholesterol (25-HC) in lung tissue in COPD model mouse

To examine the involvement of CH25H in the morbid state of COPD, the cigarette smoke-exposed mouse reflecting the morbid state of COPD used in EXAMPLE 1 was used to examine the fluctuation in the amounts of 25-HC and cholesterol in the lung tissue.

The cigarette smoke-exposed mouse had been exposed to cigarette smoke under the same conditions as in EXAMPLE 1. The number of days on which the mouse was exposed to cigarette smoke was 1, 3 or 9, and 24 hours after the final exposure, the mouse was killed by administering an excess of pentobarbital, and the lung was excised. The lung was stored at –80°C prior to measurement of the amounts of 25-HC and cholesterol.

The amounts of 25-HC and cholesterol were measured by LC/MS/MS (API 4000, Applied Biosystems/MDS Sciex) [HPLC conditions: HPLC (Shimadzu 10A), analysis column (CAPCELLPAK C18MGII, Shiseido Co., Ltd.), MS/MS

conditions: MS/MS (API 4000), Ionization mode (APCI), Ion polarity mode (positive)]. The result indicated that as the number of days on which the mouse was exposed to cigarette smoke was increased, the amount of 25-HC in the lung tissue was increased (Fig. 5A). On the other hand, no fluctuation in the amount of cholesterol was observed (Fig. 5B).

EXAMPLE 6

(1) Cloning of CH25H gene and construction of expression plasmid

Referring to human CH25H gene sequence (NM_003956) and mouse CH25H gene sequence (NM_009890), primers for cloning human and mouse CH25H full-length genes [primer 3 (SEQ ID NO: 69), primer 4 (SEQ ID NO: 70), primer 5 (SEQ ID NO: 71) and primer 6 (SEQ ID NO: 72)] (manufactured by Hokkaido System Science) were synthesized. Using these primers and lung Marathon cDNA library (Clontech) as the template for the human gene or spleen Marathon cDNA library (Clontech) as the template for the mouse gene, each full-length gene was amplified by using PyroBest polymerase and Ex-Taq polymerase (Takara Shuzo Co., Ltd.) according to its attached manual. Each PCR product was inserted into pCR BluntII TOPO vector (Invitrogen) according to its attached manual (pCRII BluntII TOPO-hCH25H and pCRII BluntII TOPO-mCH25H). Subsequently, BamHI-XhoI fragments containing hCH25H and mCH25H gene fragments were cut off from the resulting pCRII BluntII TOPO-hCH25H and pCRII BluntII TOPO-mCH25H, respectively, and then inserted into pcDNA3.1(+) vector (Invitrogen), whereby human CH25H expression plasmid (pcDNA-hCH25H) and mouse CH25H expression plasmid (pcDNA-mCH25H) were constructed.

(2) Identification of CH25H expression site

(2-1) Identification of CH25H gene expression site by in situ hybridization

The mouse CH25H gene-harboring plasmid (pCRII BluntII TOPO-mCH25H) constructed in (1) above was digested with BamHI and XhoI respectively to prepare a linear DNA having T7 promoter binding site downstream from the mouse CH25H gene and a linear DNA having SP6 promoter binding site. Using DIG RNA labeling kit (Roche Diagnostics) with these DNAs as the template, mouse CH25H antisense RNA and sense RNA were prepared according to its attached protocol. For MMP-12 gene as the control, antisense RNA and sense RNA

were prepared.

According to the method described in EXAMPLE 4, the lung was excised from a mouse exposed to cigarette smoke for 3 months, then fixed with 4% paraformaldehyde, cut into a 10 μm section with a cryostat, attached onto APS-coated slide glass and used as a sample. Hybridization was carried out by using in situ hybridization reagents (Nippon Gene) according to the attached protocol. Detection of the RNA probe was carried out by using the DIG detection kit (Roche Diagnostics).

As a result, the expression of CH25H was observed in cells distributed widely in the lung and agreed with the distribution of expression of MMP-12 used as the control, and thus the cells expressing CH25H were estimated to be alveolar macrophages.

(2-2) Identification of CH25H expression site by immunostaining

For confirming the expression site of CH25H, the expressing cells were identified by immunostaining with CH25H antibody. A peptide (SEQ ID NO: 73, manufactured by MBL) synthesized on the basis of a method described by Lund et al. (The Journal of Biological Chemistry, vol. 273, pp. 34316-34327, 1998), together with KHL, was used to immunize rabbits. The serum after 5th immunization was purified through a peptide column to prepare anti-CH25H antibody.

According to the method described in EXAMPLE 4, the lung was excised from the mouse exposed to cigarette smoke for 3 months and cut into a 10 μm section with a cryostat to prepare a sample. After drying with air, the section was fixed with Mildform for 15 minutes, then reacted with 0.3% H_2O_2/MeOH for 30 minuites, and blocked for 1 hour with Block Ace (Snowbrand Co., Ltd.). Thereafter, the sample was reacted with macrophage-recognizing anti-mouse F4/80 antibody (UK-Serotec), AlexaFluor 594-labeled anti-rat IgG antibody (Molecular Probe), anti-mouse CH25H antibody, and AlexaFluor 488-labeled anti-rabbit IgG antibody (Molecular Probe) in this order each for 30 minutes. The sample was washed with PBS/0.1% Triton X-100, then subjected to nuclear staining/encapsulation with VECTASHIELD with DAPI (manufactured by VECTOR) and observed and photographed under a fluorescence microscope.

As a result, positive cells with the anti-mouse F4/80 antibody agreed completely with positive cells with the anti-mouse CH25H antibody, thus revealing that CH25H was expressed in alveolar macrophages.

EXAMPLE 7

Involvement of 25-hydroxycholesterol in airway inflammation

(1) Influence of 25-hydroxycholesterol on production of cytokines in bronchoalveolar lavage fluid

To examine the involvement of CH25H on the morbid state of COPD, the effect of added 25-hydroxycholesterol (25-HC), i.e. CH25H reaction product, on alveolar macrophages was examined.

According to the method described in EXAMPLE 4, a mouse was exposed to cigarette smoke for 4 days, and on the next day of the final exposure, the bronchoalveolar lavage fluid was recovered according to the method described in EXAMPLE 4, and cells containing alveolar macrophages were seeded on a 96-well plate at a density of 1×10^6 cells/ml. On the next day, the cells were stimulated with LPS (10 ng/ml) and 25-HC (0.3 to 3 μg/ml) and cultured for 24 hours. According to the method described in EXAMPLE 1, total RNA was then recovered from the cells according to the attached manual, and the amounts of mRNAs of CXCL2 and IL-1β, that is, inflammatory cytokines, were quantified by the real-time quantitative PCR method. As each probe, the corresponding probe was selected from Assays on demand gene expression product (Applied Biosystems) and used. The expression level of each gene was calculated as relative expression value to rodent GAPDH by the comparative Ct value method.

As a result, the expression of CXCL2 and IL-1β was increased depending on the concentration of 25HC by co-stimulation with LPS and 25-HC, as compared with stimulation with LSP alone (Fig. 6).

(2) Production of cytokines by intratracheal administration of 25-hydroxycholesterol (25-HC)

To examine whether production of cytokines by 25-HC is observed in vivo as well, the effect of 25-HC administered to the lung of a mouse was examined.

25-HC or a control (physiological saline containing a solvent at the same concentration as used in dissolving 25-HC (10% aqueous ethanol)) was intratracheally administered at a dose of 50 μg/50 μl/mouse to C57BL/6 mice (6-week-old, male) under anesthesia with halothane, and 3, 6, 12, 24 and 48 hours after administration, the bronchoalveolar lavage fluid was recovered, and the amounts of KC and MIP-2, that is, inflammatory cytokines in the lavage fluid were

measured by a commercial ELISA kit.

As a result, a significant increase in the amounts of KC and MIP-2 was recognized 3 hours after administration of 25-HC (Fig. 7).

(3) Neutrophil infiltration by intratracheal administration of 25-hydroxhcholesterol

To examine whether the airway inflammatory reaction by 25-HC is observed in vivo as well, the effect of 25-HC administered to the lung of a mouse was examined.

25-HC or a control (physiological saline containing a solvent at the same concentration as used in dissolving 25-HC (9.5% aqueous ethanol)), or 4β-hydroxycholesterol (4β-HC), was intratracheally administered for 4 days at a dose of 50 μg/50 μl/mouse/day to C57BL/6 mice (7-week-old, male) under anesthesia with ketamine and xylazine, and on the next day, the bronchoalveolar lavage fluid was recovered, and the number of inflammatory cells was determined. As a result, significant neutrophil infiltration was recognized in the 25-HC administration group only (Fig. 8).

From these results, CH25H produces 25-HC from cholesterol in alveolar macrophage, and its product 25-HC promotes production of inflammatory cytokines such as CXCL2 and IL-1β, thereby accelerating neutrophil infiltration in the airway and advancing the morbid state of COPD.

EXAMPLE 8

Screening of compounds having an inhibitory action on CH25H

COS cells were seeded onto a 6-well plate at a density of 2×10^5 cells/well, and on the next day, 2 μg of the above human CH25H expression plasmid (pcDNA-hCH25H) was introduced by using FuGENE6 (Roche Diagnostics) according to its attached manual. The cells were further cultured for 2 days, and after the medium was exchanged with a serum-free DEM medium containing 2-hydroxypropyl-β-cyclodextrin at a concentration of 20 mg/ml, the cells were cultured at 37°C for 1 hour. Thereafter, the medium was exchanged with 5% lipopotein-poor serum-containing DMEM medium, and a test compound was added at a concentration of 10 μM. After 10 minutes, 2 μl ^{14}C-cholesterol (final concentration 0.4 μM) was added, and the culture was continued. After 24 hours, cholesterols were extracted from the culture supernatant with an equal volume of $CHCl_3$:MeOH (2:1). The organic layer was evaporated to dryness, then

re-dissolved in $CHCH_3$:MeOH (2:1) and applied onto Silica Gel 60 TLC plate (20 cm × 20 cm, manufactured by Merck). Thereafter, the sample was developed with AcOEt/Ph-Me (4:6), and the conversion of cholesterols into 25-HC was detected by BAS2000 thereby determining CH25H enzyme activity (degree of conversion). Simultaneously, the CH25H enzyme activity (degree of conversion) of converting cholesterols into 25HC in the absence of a test compound was also determined.

From these results, the degree of inhibition of CH25H enzyme activity was calculated according to the following equation:

Degree of inhibition (%) = (1 – (degree of conversion in the presence of the test compound/degree of conversion in the absence of the test compound)) × 100

The test compound showing a degree (%) of inhibition of 50% or more was selected as a compound having an inhibitory action on CH25H.

INDUSTRIAL APPLICABILITY

A protein comprising an amino acid sequence identical or substantially identical with the amino acid sequence represented by SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 30, SEQ ID NO: 32, SEQ ID NO: 34, SEQ ID NO: 36, SEQ ID NO: 38, SEQ ID NO: 40, SEQ ID NO: 42, SEQ ID NO: 44, SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 58, SEQ ID NO: 60, SEQ ID NO: 62, SEQ ID NO: 64 or SEQ ID NO: 66, and a polynucleotide encoding the protein, are useful for example as diagnostic markers etc. for respiratory diseases [for example, chronic obstructive pulmonary disease (for example, chronic bronchitis, pulmonary emphysema), diffuse panbronchiolitis, bronchial asthma, cystic fibrosis, hypersensitive pneumonia, pulmonary fibrosis etc.], chronic obstructive pulmonary disease (for example, chronic bronchitis, pulmonary emphysema), diffuse panbronchiolitis, bronchial asthma, cystic fibrosis, hypersensitive pneumonia, pulmonary fibrosis etc.] [sic].

A protein comprising an amino acid sequence identical or substantially identical with the amino acid sequence represented by SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 30, SEQ ID NO: 32, SEQ

ID NO: 34, SEQ ID NO: 36, SEQ ID NO: 38, SEQ ID NO: 40, SEQ ID NO: 42, SEQ ID NO: 44, SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 58, SEQ ID NO: 60 or SEQ ID NO: 62, a polynucleotide encoding the protein, an inhibitor obtained by screening using e.g. an antibody to the protein, a neutralizing antibody inhibiting the activity of the protein, and an antisense polynucleotide to the above polynucleotide, can be used for example as prophylactic/therapeutic agents for respiratory diseases [for example, chronic obstructive pulmonary disease (chronic bronchitis, pulmonary emphysema), diffuse panbronchiolitis, bronchial asthma, cystic fibrosis, hypersensitive pneumonia, pulmonary fibrosis etc.].

A protein comprising an amino acid sequence identical or substantially identical with the amino acid sequence represented by SEQ ID NO: 64 or SEQ ID NO: 66, a polynucleotide encoding the protein, a promoter obtained by screening using e.g. an antibody to the protein, an antibody promoting the activity of the protein, the above protein, and the above polynucleotide can be used for example as prophylactic/therapeutic agents for respiratory diseases [for example, chronic obstructive pulmonary disease (chronic bronchitis, pulmonary emphysema), diffuse panbronchiolitis, bronchial asthma, cystic fibrosis, hypersensitive pneumonia, pulmonary fibrosis etc.].